BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

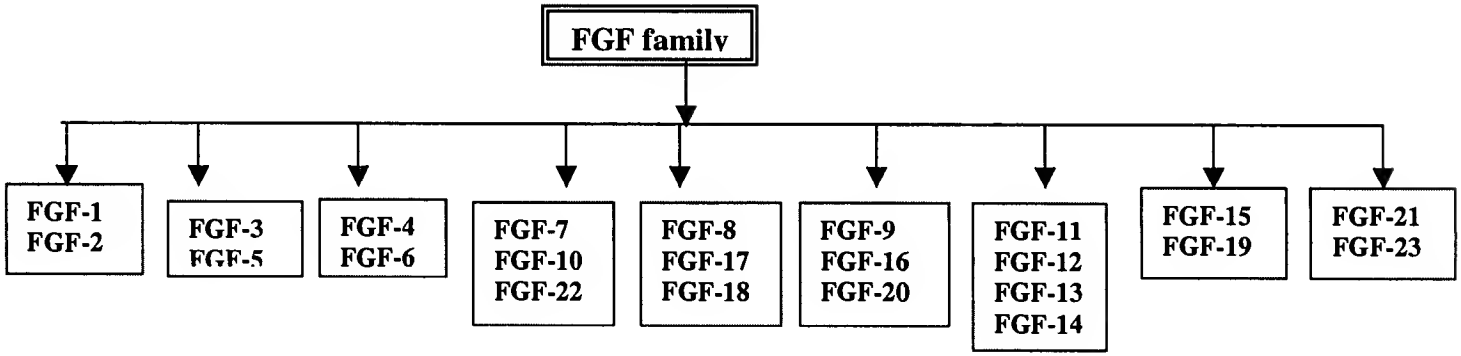
Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

Appendix I. Evolutionary Relationship of FGF superfamily



Appendix II. FGF receptor profile

FGF family	FGFR1 IIIb	FGFR1 IIIc	FGFR2 IIIb	FGFR2 IIIc	FGFR3 IIIb	FGFR3 IIIc	FGFR4
FGF2	+	++	-	+	-	++	++
FGF3	+	-	+	-	-	-	-
FGF5	-	+	-	+/-	-	-	-
FGF7 (KGF)	+/-	-	++	-	-	-	-
FGF10 (KGF2)	+	-	++	-	-	-	N/A
FGF22	+	-	++	-	-	-	-
FGF9	-	+/-	-	++	+	++	+
FGF20	N/A	+	++	++	N/A	++	+
FGF4	+/-	++	+/-	++	-	++	++
FGF8	-	-	-	+/-	-	+	+
FGF23	-	-	-	+	-	++	-

Protein family review

Fibroblast growth factors

David M Ornitz* and Nobuyuki Itoh†

Addresses: *Department of Molecular Biology and Pharmacology, Washington University Medical School, 660 S. Euclid Avenue, St. Louis, MO 63110, USA. †Department of Genetic Biochemistry, Kyoto University Graduate School of Pharmaceutical Sciences, Yoshida-Shimoadachi, Sakyo, Kyoto 606-8501, Japan.

Correspondence: David M Ornitz. E-mail: dornitz@molecool.wustl.edu

Published: 9 March 2001

Genome ***Biology*** 2001, **2(3)**:reviews3005.1–3005.12

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2001/2/3/reviews/3005

Summary

Fibroblast growth factors (FGFs) make up a large family of polypeptide growth factors that are found in organisms ranging from nematodes to humans. In vertebrates, the 22 members of the FGF family range in molecular mass from 17 to 34 kDa and share 13-71% amino acid identity. Between vertebrate species, FGFs are highly conserved in both gene structure and amino-acid sequence. FGFs have a high affinity for heparan sulfate proteoglycans and require heparan sulfate to activate one of four cell-surface FGF receptors. During embryonic development, FGFs have diverse roles in regulating cell proliferation, migration and differentiation. In the adult organism, FGFs are homeostatic factors and function in tissue repair and response to injury. When inappropriately expressed, some FGFs can contribute to the pathogenesis of cancer. A subset of the FGF family, expressed in adult tissue, is important for neuronal signal transduction in the central and peripheral nervous systems.

Gene organization and evolutionary history

Gene organization

The prototypical *Fgf* genes contain three coding exons (Figure 1), with exon 1 containing the initiation methionine, but several *Fgf* genes (for example, *Fgf2* and *Fgf3*) have additional 5' transcribed sequence that initiates from upstream CUG codons [1,2]. The size of the coding portion of *Fgf* genes ranges from under 5 kb (in *Fgf3* and *Fgf4*) to over 100 kb (in *Fgf12*). In several *Fgf* subfamilies, exon 1 is subdivided into between two and four alternatively spliced subexons (denoted 1A-1D in the case of *Fgf8*). In these *Fgf* genes, a single initiation codon (ATG) in exon 1A is used. This gene organization is conserved in humans, mouse and zebrafish, but its functional consequences are poorly understood. Other subfamilies of *Fgf*s (such as *Fgf11-14*) have alternative amino termini, which result from the use of alternative 5' exons. It is not known whether a common 5' untranslated exon splices to these exons or whether alternative promoter and regulatory sequences are used.

Most *Fgf* genes are found scattered throughout the genome. In human, 22 *FGF* genes have been identified and the chromosomal locations of all except *FGF16* are known (Table 1) [3-7]. Several human *FGF* genes are clustered within the genome. *FGF3*, *FGF4* and *FGF19* are located on chromosome 11q13 and are separated by only 40 and 10 kb, respectively; *FGF6* and *FGF23* are located within 55 kb on chromosome 12p13; and *FGF17* and *FGF20* map to chromosome 8p21-p22. These gene locations indicate that the *FGF* gene family was generated both by gene and chromosomal duplication and translocation during evolution. Interestingly, a transcriptionally active portion of human *FGF7*, located on chromosome 15q13-q22, has been amplified to about 16 copies, which are dispersed throughout the human genome [8].

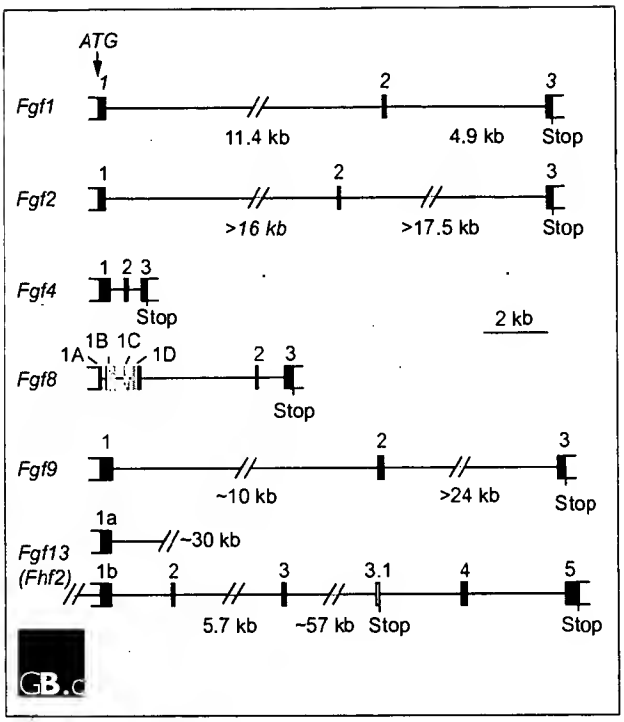


Figure 1
Gene structure of selected members of the *Fgf* family. Only the portion of each gene containing coding exons is shown. Constitutively expressed exons are in black; alternatively spliced exons are in gray. *Fgfs 1, 2, 4* and *9* contain the prototypic three-exon organization. For *Fgf1*, 5' untranslated exons are not shown; inclusion of these exons extends the gene by approximately 69 kb [78]. *Fgf8* is an example of a gene with 5' alternative splicing, and *Fgf13* demonstrates alternatively used 5' exons separated by over 30 kb. References: *Fgf1* [78]; *Fgf2* [79]; *Fgf4* [80]; *Fgf8* [52]; *Fgf9* [81]; *Fgf13* [76].

In the mouse, there are at least 22 *Fgf* genes [3,9], and the locations of 16 have been identified (Table 1). Many of the mouse *Fgf* genes are scattered throughout the genome, but as in the human, *Fgf3*, *Fgf4* and *Fgf19* are closely linked (within 80 kb on chromosome 7F) and *Fgf6* and *Fgf23* are closely linked on chromosome 6F3-G1.

Evolutionary history

Fgfs have been identified in both invertebrates and vertebrates [3]. Interestingly, an *Fgf*-like gene is also encoded in the nuclear polyhedrosis virus genome [10]. *Fgf*-like sequences have not been found in unicellular organisms such as *Escherichia coli* and *Saccharomyces cerevisiae*. Although the *Drosophila* and *Caenorhabditis elegans* genomes have been sequenced, only one *Fgf* gene (*branchless*) has been identified in *Drosophila* [11] and two (*egl-17* and *let-756*) have been identified in *C. elegans* [12,13], in contrast to the large number of *Fgf* genes identified in vertebrates. The evolutionary relationship between invertebrate and vertebrate *Fgfs* is shown in Figure 2a.

The *Fgf* gene expansion has been hypothesized to be coincident with a phase of global gene duplications that took place during the period leading to the emergence of vertebrates [14]. Across species, most orthologous FGF proteins are highly conserved and share greater than 90% amino-acid sequence identity (except human FGF15 and mouse Fgf19; see below). To date, four *Fgfs* (*Fgf3, 8, 17* and *18*) have been identified in zebrafish, seven (*Fgf3, Fgf(i), Fgf(ii), Fgf8, 9* and *20*) in *Xenopus* (*Fgf(i) and Fgf(ii)* are most closely related to *Fgf4* and *Fgf6* [15]) and seven (*Fgf2, 4, 8, 12, 14, 18* and *19*) in chicken [3].

The apparent evolutionary relationships of the 22 known human FGFs are shown in Figure 2b. Vertebrate FGFs can be classified into several subgroups or subfamilies. Members of a subgroup of FGFs share increased sequence similarity and biochemical and developmental properties. For example, members of the FGF8 subfamily (FGF8, FGF17, and FGF18) have 70-80% amino acid sequence identity, similar receptor-binding properties and some overlapping sites of expression (for example, the midbrain-hindbrain junction) [16,17]. Members of FGF subgroups are not closely linked in the genome, however, indicating that the subfamilies were generated by gene-translocation or by genome-duplication events, not by local duplication events.

Human *FGF15* and mouse *Fgf19* have not been identified. Human *FGF19* is evolutionarily most closely related to mouse *Fgf15* (51% amino acid identity; Figure 2b) [18] and both the human *FGF19* and mouse *Fgf15* genes are closely linked to the human and mouse *Fgf3* and *Fgf4* genes on orthologous regions of human chromosome 11q13 and mouse chromosome 7F (N.I., unpublished observations). These findings indicate that human *FGF19* may be the human ortholog of mouse *Fgf15*. Because all other *Fgf* orthologs share greater than 90% amino acid identity, it remains possible that the true orthologs of these genes have not been identified, have been lost or have diverged during vertebrate evolution.

Characteristic structural features

FGFs range in molecular weight from 17 to 34 kDa in vertebrates, whereas the *Drosophila* FGF is 84 kDa. Most FGFs share an internal core region of similarity, with 28 highly conserved and six identical amino-acid residues [19]. Ten of these highly conserved residues interact with the FGF receptor (FGFR) [20]. Structural studies on FGF1 and FGF2 identify 12 antiparallel β strands in the conserved core region of the protein (Figure 3) [21,22]. FGF1 and FGF2 have a β trefoil structure that contains four-stranded β sheets arranged in a triangular array (Figure 3b; reviewed in [23]). Two β strands (strands β10 and β11) contain several basic amino-acid residues that form the primary heparin-binding site on FGF2. Regions thought to be involved in receptor binding are distinct from regions that bind heparin (Figure 3) [21-24].

Table 1

Chromosomal localizations of FGFs in human and mouse

Human		Mouse		References	Accession numbers	
Gene	Location	Gene	Location		Human	Mouse
FGF1	5q31	*Fgf1*	18	[82,83]	X65778, E03692, E04557	U67610, M30641
FGF2	4q26-27	*Fgf2*	3A2-B	[84,85]	E05628, M27968	M30644, AF065903, AF065904, AF065905
FGF3	11q13	*Fgf3*	7F	[86-88]	X14445	Y00848
FGF4	11q13.3	*Fgf4*	7F	[87,89]	E03343	M30642
FGF5	4q21	*Fgf5*	5E1-F	[85,90]	M37825	M30643
FGF6	12p13	*Fgf6*	6F3-G1	[91,92]	X63454	M92416
FGF7	15q15-21.1	*Fgf7*	2F-G	[93,94]	M60828	Z22703
FGF8	10q24	*Fgf8*	19C3-D	[54,95]	U36223, U56978	Z48746
FGF9	13q11-q12	*Fgf9*	14D	[81,96,97]	D14838	U33535, D38258
FGF10	5p12-p13	*Fgf10*	13A3-A4	[98,99]	AB002097	D89080
FGF11 (FHF3)	17p13.1	*Fgf11*	-	[100]	U66199	U66203
FGF12 (FHF1)	3q28	*Fgf12*	16B1-B3	[31,100-102]	U66197	U66201
FGF13 (FHF2)	Xq26	*Fgf13*	X	[31,76,103]	U66198	U66202, AF020737
FGF14 (FHF4)	13q34	*Fgf14*	14	[31]	U66200	U66204
-		*Fgf15**	7F	(N.I., unpublished observations)		AF007268
FGF16	-	*Fgf16*	-		AB009391	AB049219
FGF17	8p21	*Fgf17*	14	[104]	AB009249	AB009250
FGF18	5q34	*Fgf18*	-	[105]	AB007422, AF075292	AB004639, AF075291
*FGF19**	11q13.1	-		[106]	AB018122, AF110400	
FGF20	8p21.3-p22	*Fgf20*	-	[27,107]	AB030648, AB044277	AB049218
FGF21	19q13.1-qter	*Fgf21*	-	[108]	AB021975	AB025718
FGF22	19p13.3	*Fgf22*	-	[109]	AB021925	AB036765
FGF23	12p13.3	*Fgf23*	6F3-G1	[7,75] (N.I., unpublished)	AB037973, AF263537	AB037889, AF263536

*Human *FGF19* and mouse *Fgf15* may be orthologous genes.

Localization and function

Localization

Subcellular localization and secretion

Most FGFs (FGFs 3-8, 10, 15, 17-19, and 21-23) have amino-terminal signal peptides and are readily secreted from cells. FGFs 9, 16 and 20 lack an obvious amino-terminal signal peptide but are nevertheless secreted [25-27]. FGF1 and FGF2 also lack signal sequences, but, unlike FGF9, are not secreted; they can, however, be found on the cell surface and within the extracellular matrix. FGF1 and FGF2 may be released from damaged cells or could be released by an exocytotic mechanism that is independent of the endoplasmic-reticulum-Golgi pathway [28]. FGF9 has been shown to contain a non-cleaved amino-terminal hydrophobic sequence that is required for secretion [29,30]. A third subset of FGFs (FGF11-14) lack signal sequences and are thought to remain intracellular [31-34]. It is not known whether these FGFs interact with known FGFRs or function in a receptor-independent manner within the cell. FGF2 and FGF3 have high-molecular-weight forms that arise from initiation from upstream CUG codons [2,14,35]. The additional amino-terminal sequence in these proteins contains nuclear-localization signals, and the proteins can be found in the nucleus; the biological function of nuclear-localized FGF is unclear.

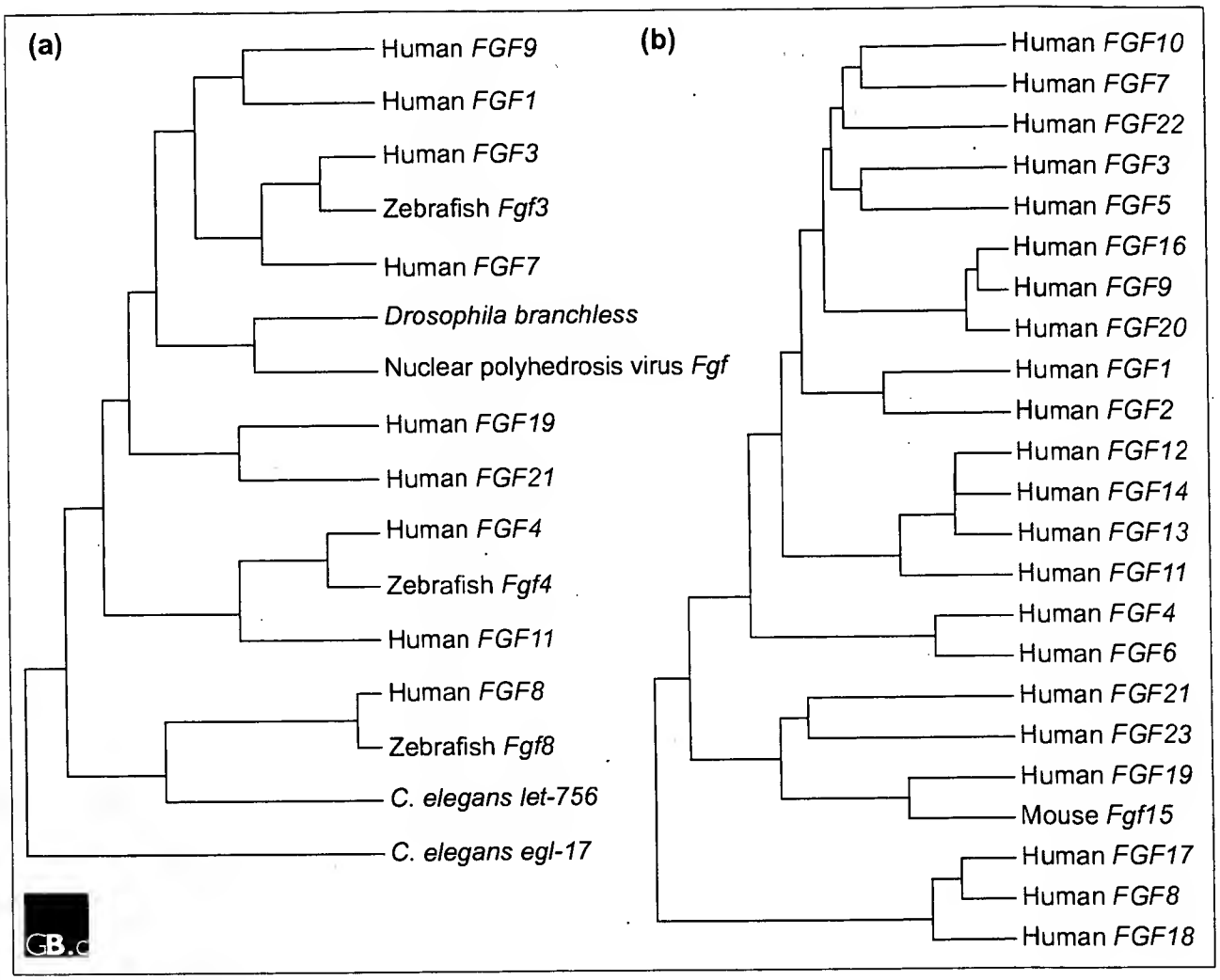


Figure 2
Evolutionary relationships within the FGF family. **(a)** Apparent evolutionary relationships between FGFs from vertebrates, invertebrates and a virus. Amino-acid sequences of nine representative FGFs were chosen from human and compared with FGFs from *Drosophila*, *C. elegans*, zebrafish and *Autographa californica* nuclear polyhedrosis virus. **(b)** Apparent evolutionary relationships of the 22 known human and murine FGFs. Sequences were aligned using Genetyx sequence analysis software and trees were constructed from the alignments using the neighbor-joining method.

Developmental expression patterns and function

The 22 members of the mammalian FGF family are differentially expressed in many, if not all, tissues, but the patterns and timing of expression vary. Subfamilies of FGFs tend to have similar patterns of expression, although each FGF also appears to have unique sites of expression. Some FGFs are expressed exclusively during embryonic development (for example, *Fgf3, 4, 8, 15, 17* and *19*), whereas others are expressed in embryonic and adult tissues (for example, *Fgf1, 2, 5-7, 9-14, 16, 18,* and *20-23*).

Function

The expression patterns of FGFs (see above) suggest that they have important roles in development. FGFs often signal directionally and reciprocally across epithelial-mesenchymal boundaries [36]. The integrity of these signaling pathways requires extremely tight regulation of FGF activity and receptor specificity. For example, in vertebrate limb development, mesenchymally expressed *Fgf10* in the lateral-plate mesoderm induces the formation of the overlying apical ectodermal ridge; the ridge subsequently expresses *Fgf8*, which signals back to the underlying mesoderm [37]. This directional signaling initiates feedback loops and, along with other signaling molecules, regulates the outgrowth and patterning of the limb. Importantly, the differential expression of the alternative splice forms of the receptors in the apical ectodermal ridge and underlying-mesoderm is such as to limit or prevent autocrine signaling within a given compartment.

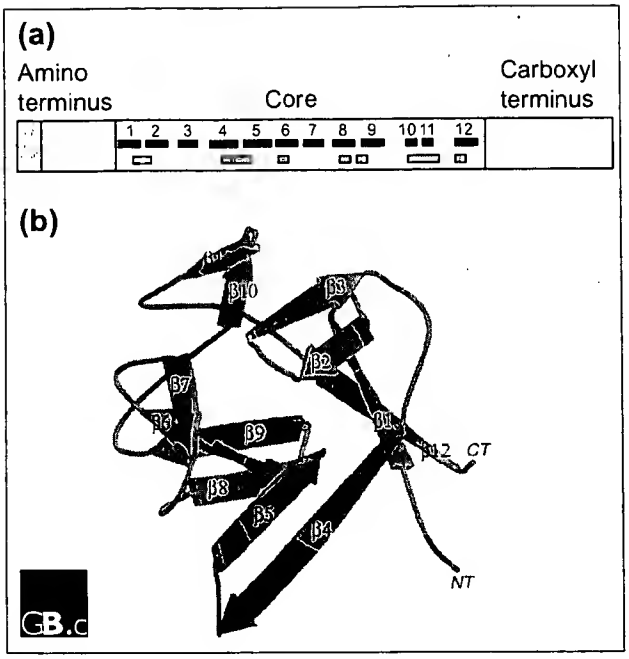


Figure 3
(a) Structural features of the FGF polypeptide. The amino terminus of some FGFs contains a signal sequence (shaded). All FGFs contain a core region that contains conserved amino-acid residues and conserved structural motifs. The locations of β strands within the core region are numbered and shown as black boxes. The heparin-binding region (pink) includes residues in the loop between β strands 1 and 2 and in β strands 10 and 11. Residues that contact the FGFR are shown in green (the region contacting Ig-domain 2 of the receptor), blue (contacting Ig-domain 3) and red (contacting the alternatively spliced region of Ig-domain 3). Amino-acid residues that contact the linker region are shown in gray [20]. **(b)** Three-dimensional structure of FGF2, a prototypical member of the FGF family. A ribbon diagram of FGF2 is shown; β strands are labeled 1-12 and regions of contact with the FGFR and heparin are color-coded as in (a) [22,24]. Image provided by M. Mohammadi.

Studies of the biochemical activities of FGFs have focused on the specificity of interactions between FGFs and FGFRs, on factors that affect the stability of FGFs and on the composition and mechanism of the active FGF-FGFR signaling complex.

Specificity of FGFs for FGF receptors

The FGFR tyrosine kinase receptors contain two or three immunoglobulin-like domains and a heparin-binding sequence [38-40]. Alternative mRNA splicing of the FGFR gene specifies the sequence of the carboxy-terminal half of immunoglobulin-domain III, resulting in either the IIIb or the IIIc isoform of the FGFR [41-43]. This alternative-splicing event is regulated in a tissue-specific manner and dramatically affects ligand-receptor binding specificity [44-48]. Exon IIIb is expressed in epithelial lineages and exon IIIc tends to be expressed in mesenchymal lineages [44,46-48]. *In vitro* patterns of binding specificity have been determined for each splice form of FGFR1-3 and for FGFR4, which is not alternatively spliced [49-51]. Ligands specific for these receptor splice forms are expressed in adjacent tissues, resulting in directional epithelial-mesenchymal signaling. For example, epithelially expressed FGFR2b (that is, FGFR2 IIIb isoform) can be activated by FGF7 and FGF10, ligands produced in mesenchymal tissue [49-51]. These ligands show no activity towards mesenchymally expressed FGFR2c. Conversely, FGF8 is expressed in epithelial tissue and activates FGFR2c but shows no activity towards FGFR2b ([49,52] and our unpublished observations). Notably, FGF8 expression is often restricted to epithelial tissue such as the apical ectodermal ridge of the developing limb bud [53,54].

Interaction with heparin or heparan sulfate proteoglycans

An important feature of FGF biology involves the interaction between FGF and heparin or heparan sulfate (HS) proteoglycan (HSPG) [19]. These interactions stabilize FGFs to thermal denaturation and proteolysis and may severely limit their diffusion and release into interstitial spaces [55,56]. FGFs must saturate nearby HS-binding sites before exerting an effect on tissue further away, or else must be mobilized by heparin/HS-degrading enzymes. The interaction between FGFs and HS results in the formation of dimers and higher-order oligomers [57-59]. Although the biologically active form of FGF is poorly defined, it has been established that heparin is required for FGF to effectively activate the FGFR in cells that are deficient in or unable to synthesize HSPG or in cells pretreated with heparin/HS-degrading enzymes or inhibitors of sulfation [60-62]. Genetic studies have also shown that mutations in enzymes involved in HS biosynthesis affect FGF signaling pathways during development [19,63]. Additional studies have shown that heparin and/or HS act to increase the affinity and half-life of the FGF-FGFR complex (reviewed in [40,64]).

A minimal complex containing one FGF molecule per FGFR can form in the absence of HS [24]. Structural studies suggest that HS may bridge FGF2 and the FGFR by binding to a groove formed by the heparan-binding sites of both the ligand and the receptor [24,65]. Binding studies with soluble chimeric FGFRs have identified a second potential FGF-binding site that, in some cases, can interact cooperatively with the primary FGF-binding site [66].

Important mutants

Many members of the *Fgf* family have been disrupted by homologous recombination in mice. The phenotypes range from very early embryonic lethality to subtle phenotypes in adult mice. The major phenotypes observed in *Fgf* knockout mice are shown in Table 2. Because FGFs within a subfamily have similar receptor-binding properties and overlapping patterns of expression, functional redundancy is likely to occur. This has been demonstrated for *Fgf17* and *Fgf8*, which cooperate to regulate neuroepithelial proliferation in the mid-brain-hindbrain junction [17]. In the case of *Fgf* knockouts

resulting in early lethality, other functions later in development will need to be addressed by constructing conditional alleles that can be targeted at specific times and places in development. For example, *Fgf8* $^{-/-}$ mice die by embryonic day 9.5 [67]. A conditional allele for *Fgf8* targeted to the apical ectodermal ridge has been used to demonstrate an essential role for *Fgf8* in early limb development [68,69].

Several mutations in *Fgf* genes have been identified in *C. elegans*, *Drosophila*, zebrafish, mouse and human. The *C. elegans* gene *egl-17* is required for sex myoblast migration [12], and a null allele of *let-756* causes developmental arrest of the early larva [13]. The *Drosophila branchless* gene is required for tracheal branching and cell migration [11]. In zebrafish, *acerebellar (ace)* embryos lack the cerebellum and the midbrain-hindbrain boundary organizer. The *ace* gene encodes the zebrafish homolog of *Fgf8* [70]. Interestingly, zebrafish *aussicht* mutant embryos, which overexpress *Fgf8*, also have defects in development of the central nervous system [71].

In the mouse, the *angora* mutation, which affects hair growth, was found to be allelic with *Fgf5* [72]. A mouse mutant with a Crouzon-syndrome-like craniofacial dysmorphology phenotype was found to result from an insertional mutation in the *Fgf3/Fgf4* locus [73]. Recently, positional cloning of the autosomal dominant hypophosphataemic rickets gene identified missense mutations in human FGF23 [74]. A recent paper demonstrates that this disease is caused by a gain-of-function mutation [75]. The chromosomal location (Xq26) and tissue-specific expression pattern of *Fgf13* (also called *Fhf2*) suggests that it may be a candidate gene for Borjeson-Forssman-Lehmann syndrome, an X-linked mental retardation syndrome [76].

Frontiers

Issues most studied

FGFs have been intensely studied for nearly 30 years. Most of the early work focused on the mechanisms that regulate stability, secretion, export and interactions with heparin and on the mechanisms and consequences of signal transduction in various types of cells. More recent work has focused on the mechanisms regulating receptor specificity and receptor activation, the structure of the FGF-FGFR-HS complex, and the identification of new members of the FGF family. Functional studies have begun to address the role of FGFs in cell biology, development and physiology. Initial studies focused on the regulation of cell proliferation, migration and differentiation; more recent work has addressed the negative

Table 2

FGF knockout mice

Gene	Survival of null mutant*	Phenotype	References
Fgf1	Viable	None identified	[110]
Fgf2	Viable	Mild cardiovascular, skeletal, neuronal	[110-114]
Fgf3	Viable	Mild inner ear, skeletal (tail)	[115]
Fgf4	Lethal, E4-5	Inner cell mass proliferation	[116]
Fgf5	Viable	Long hair, angora mutation	[72]
Fgf6	Viable	Subtle, muscle regeneration	[117-119]
Fgf7	Viable	Hair follicle growth, ureteric bud growth	[120,121]
Fgf8	Lethal, E7	Gastrulation defect, CNS development, limb development	[67,70,122,123]
Fgf9	Lethal, P0	Lung mesenchyme, XY sex reversal	[124]; (J.S. Colvin *et al.*, personal communication)
Fgf10	Lethal, P0	Development of multiple organs, including limb, lung, thymus, pituitary	[125-127]
Fgf12 (Fhf1)	Viable	Neuromuscular phenotype	(J. Schoorlemmer and M. Goldfarb, personal communication)
Fgf14 (Fhf4)	Viable	Neurological phenotypes	(Q. Wang, personal communication)
Fgf15	Lethal, E9.5	Not clear	(J.R. McWhirter, personal communication)
Fgf17	Viable	Cerebellar development	[17]
Fgf18	Lethal, P0	Skeletal development	(N. Ohbayashi, Z. Liu, personal communication)

*E, embryonic day; P, postnatal day.

effect of FGFs and FGFRs on proliferation of some cell types, which was surprising as FGFs were thought to promote proliferation. *In vitro* studies have now been complemented by gene targeting in mice. The knockout approach has been fairly successful in identifying primary phenotypes but will be challenged by the need to address redundancy amongst the 22 FGFs and to study their developmental and physiological functions after the point of lethality of the null allele.

Unresolved questions

A major unresolved question concerns the mechanism(s) regulating FGF activity *in vivo* in the presence of cell-surface and extracellular-matrix HSPG. Current hypotheses predict that tissue-specific heparan fragments of defined sequence (and particularly of defined sulfation pattern) will differentially regulate FGFs by controlling their diffusion in the extracellular matrix and their ability to activate specific receptors [77]. These issues will be resolved by determining the sequence of tissue-specific HS and by demonstrating whether specific HS sequences can modulate the binding specificity of FGFs beyond that determined by the specific FGFR and its alternative splice form in the presence of heparin.

A second area of research will aim to elucidate the developmental roles of all the FGFs, first alone and then in various combinations. This will include determining whether a single FGF with a defined developmental function interacts with one or multiple FGFRs. A third major frontier will be to elucidate the physiological roles of FGFs that are expressed in adult tissues. This will again involve testing combinations of FGFs in cases in which knockouts are viable and designing conditional alleles in cases of embryonic lethality. Major areas being considered include neuronal and cardiovascular physiology, neuronal regeneration and homeostasis and tissue repair.

The last major frontier will be to elucidate the primary roles of FGFs in genetic diseases and cancer. Several FGFs were initially cloned from human and animal tumors. Future work will be required to determine whether FGF activation is itself an etiological agent in primary human tumors or whether it is a progression factor in the pathogenesis of cancer. As functional roles for FGFs are elucidated in embryonic development, it is expected that various human birth defects and genetic diseases will be attributed to mutations in *Fgf* genes. These studies will probably lead to the development of pharmacogenetic agents to treat these diseases. Because a large number of skeletal diseases are caused by mutations in *Fgfr* genes, it is anticipated that mutations in some *Fgf* genes will also be involved in skeletal pathology.

Acknowledgements

This work was supported by NIH grants CA60673 and HD3S692 and by a grant from the American Heart Association (to D.M.O.) and by the Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan and a grant from the Human Frontier Science Program, France (to N.I.).

References

1. Kiefer P, Acland P, Pappin D, Peters G, Dickson C: **Competition between nuclear localization and secretory signals determines the subcellular fate of a single CUG-initiated form of FGF3.** *EMBO J* 1994, 13:4126-4136.
   This is one of first papers to show that an FGF can initiate from an upstream CUG codon and can be localized in the nucleus.
2. Arnaud E, Touriol C, Boutonnet C, Gensac MC, Vagner S, Prats H, Prats AC: **A new 34-kilodalton isoform of human fibroblast growth factor 2 is cap dependently synthesized by using a non-AUG start codon and behaves as a survival factor.** *Mol Cell Biol* 1999, 19:S0S-S14.
   Identification of a high-molecular-weight form of FGF2 that initiates from an upsteam CUG codon and is localized in the nucleus.
3. **GenBank** [http://www.ncbi.nlm.nih.gov/Genbank/index.html]
   DNA sequences of FGFs listed in Table 1 can be accessed through this website.
4. **HUGO Gene Nomenclature Database**
   [http://www.gene.ucl.ac.uk/nomenclature/]
   This database contains nomenclature and mapping information for human genes.
5. **Cytokine Family cDNA Database**
   [http://cytokine.medic.kumamoto-u.ac.jp/CFC/FGF/FGF.html]
   This database contains nomenclature and mapping information for the FGF family.
6. **LocusLink** [http://ncbi.nlm.nih.gov/LocusLink/]
   This database contains updated mapping information for the FGF family with links to a variety of other databases.
7. Yamashita T, Yoshioka M, Itoh N: **Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain.** *Biochem Biophys Res Commun* 2000, 277:494-498.
   FGF23 is the most recent reported member of the FGF family. This paper summarizes the chromosomal localization and apparent evolutionary relationships of human FGFs.
8. Kelley MJ, Pech M, Seuanez HN, Rubin JS, O'Brien SJ, Aaronson SA: **Emergence of the keratinocyte growth factor multigene family during the great ape radiation.** *Proc Natl Acad Sci USA* 1992, 89:9287-9291.
   This paper identifies a human multigene family composed of amplified and dispersed *FGF7* gene copies and suggests models for the evolution of the great apes and humans.
9. **Mouse Genome Informatics** [http://www.informatics.jax.org]
   This website provides information on mouse gene sequences, map position and function.
10. Ayres MD, Howard SC, Kuzio J, Lopez-Ferber M, Possee RD: **The complete DNA sequence of *Autographa californico* nuclear polyhedrosis virus.** *Virology* 1994, 202:S86-60S.
    The complete nucleotide sequence of the genome of the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) revealed that this genome encodes an FGF-like protein.
11. Sutherland D, Samakovlis C, Krasnow MA: **branchless encodes a *Drosophila* FGF homolog that controls tracheal cell migration and the pattern of branching.** *Cell* 1996, 87:1091-1101.
    This paper identifies a *Drosophila* homolog (*branchless, bnl*) of mammalian FGFs, which activates the breathless FGF receptor tyrosine kinase. This FGF pathway regulates tracheal branching patterns by guiding tracheal cell migration during primary branch formation and by activating cytoplasmic branching at the ends of primary branches.
12. Burdine RD, Chen EB, Kwok SF, Stern MJ: **egl-17 encodes an invertebrate fibroblast growth factor family member required specifically for sex myoblast migration in *Caenorhabditis elegans*.** *Proc Natl Acad Sci USA* 1997, 94:2433-2437.
    The *C. elegans* gene *egl-17* is a member of the *Fgf* family and one of the first known functional invertebrate FGFs. EGL-17 acts as a ligand for EGL-15 (FGFR) to regulate sex myoblast migration.
13. Roubin R, Naert K, Popovici C, Vatcher G, Coulier F, Thierry-Mieg J, Pontarotti P, Birnbaum D, Baillie D, Thierry-Mieg D: **let-756, a *C. elegans* Fgf essential for worm development.** *Oncogene* 1999, 18:6741-6747.
    Null mutations in *C. elegans let-756* lead to developmental arrest in early larval stages; *let-756* is thus an essential gene for *C. elegans* development.
14. Coulier F, Pontarotti P, Roubin R, Hartung H, Goldfarb M, Birnbaum D: **Of worms and men: an evolutionary perspective on the**

fibroblast growth factor (FGF) and FGF receptor families. *J Mol Evol* 1997, **44**:43-56.
This review compares FGF and FGF receptor sequences in vertebrates and nonvertebrates and concludes that the FGF and FGF receptor families have evolved through phases of gene duplications, one of which may have coincided with the emergence of vertebrates. The review includes all four *Fgfrs* and 11 *Fgfs*.

15. Isaacs HV, Tannahill D, Slack JM: **Expression of a novel FGF in the *Xenopus* embryo. A new candidate inducing factor for mesoderm formation and anteroposterior specification.** Development 1992, **114**:711-720.
Identification of novel *Xenopus* FGFs.
16. Maruoka Y, Ohbayashi N, Hoshikawa M, Itoh N, Hogan BLM, Furuta Y: **Comparison of the expression of three highly related genes, *Fgf8*, *Fgf17* and *Fgf18*, in the mouse embryo.** *Mech Dev* 1998, **74**:175-177.
This paper compares the expression pattern of the *Fgf8* subfamily which includes *Fgf8*, *17* and *18*. Expression is examined during early development and in the developing brain and limbs.
17. Xu J5, Liu ZH, Ornitz DM: **Temporal and spatial gradients of Fgf8 and Fgf17 regulate proliferation and differentiation of midline cerebellar structures.** *Development* 2000, **127**:1833-1843.
This is the first paper to demonstrate cooperation between two *Fgfs* during vertebrate development.
18. Nishimura T, Utsunomiya Y, Hoshikawa M, Ohuchi H, Itoh N: **Structure and expression of a novel human FGF, FGF-19, expressed in the fetal brain.** *Biochim Biophys Acto* 1999, **1444**:148-151.
Identification of human *FGF19* as a possible homolog of mouse *Fgf15*. Both of these genes are most closely related to *FGFs* 21 and 23.
19. Ornitz DM: **FGFs, heparan sulfate and FGFRs: complex interactions essential for development.** *BioEssays* 2000, **22**:108-112.
This review article addresses the developmental implications of the interactions between FGFs, FGFRs and heparan sulfate proteoglycans. The paper by Lin *et al.* [63], which demonstrates that heparan sulfate is essential for FGF function during *Drosophila* development, is highlighted.
20. Plotnikov AN, Hubbard 5R, 5chlessinger J, Mohammadi M: **Crystal structures of two FGF-FGFR complexes reveal the determinants of ligand-receptor specificity.** *Cell* 2000, **101**:413-424.
The analysis of two different ligand-receptor crystal structures is used to determine elements that regulate ligand-binding specificity.
21. Zhu X, Komiya H, Chirino A, Faham S, Fox GM, Arakawa T, Hsu BT, Rees DC: **Three-dimensional structures of acidic and basic fibroblast growth factors.** *Science* 1991, **251**:90-93.
This paper presents one of the first structures of FGF1 and FGF2.
22. Eriksson AE, Cousens LS, Weaver LH, Matthews BW: **Three-dimensional structure of human basic fibroblast growth factor.** *Proc Natl Acod Sci USA* 1991, **88**:3441-3445.
This paper presents one of the first structures of FGF2.
23. Faham S, Linhardt RJ, Rees DC: **Diversity does make a difference: fibroblast growth factor-heparin interactions.** *Curr Opin Struct Biol* 1998, 8:578-586.
A review that models the formation of an FGF-FGFR-proteoglycan complex, based on the structure of FGF in complex with fragments of heparin.
24. Plotnikov AN, Schlessinger J, Hubbard SR, Mohammadi M: **Structural basis for FGF receptor dimerization and activation.** *Cell* 1999, **98**:641-650.
Crystal structure of FGF2 complexed with FGFR1 showing extensive interaction between immunoglobulin domains 2 and 3 and the interdomain linker of the FGFR.
25. Miyake A, Konishi M, Martin FH, Hernday NA, Ozaki K, Yamamoto S, Mikami T, Arakawa T, Itoh N: **Structure and expression of a novel member, FGF-16, of the fibroblast growth factor family.** *Biochem Biophys Res Commun* 1998, **243**:148-152.
This paper identifies *Fgf16* as a homolog of *Fgf9*.
26. Miyamoto M, Naruo K, Seko C, Matsumoto 5, Kondo T, Kurokawa T: **Molecular cloning of a novel cytokine cDNA encoding the ninth member of the fibroblast growth factor family, which has a unique secretion property.** *Mol Cell Biol* 1993, **13**:4251-4259.
This paper identifies FGF9 as a new member of the FGF family without a classical signal peptide.
27. Ohmachi S, Watanabe Y, Mikami T, Kusu N, Ibi T, Akaike A, Itoh N: **FGF-20, a novel neurotrophic factor, preferentially expressed in the substantia nigra pars compacta of rat brain.** *Biochem Biophys Res Commun* 2000, **277**:355-360.
This paper identifies *Fgf20* as a homolog of *Fgf9* and *Fgf16*.
28. Mignatti P, Morimoto T, Rifkin DB: **Basic fibroblast growth factor, a protein devoid of secretory signal sequence, is released by cells via a pathway independent of the endoplasmic reticulum-Golgi complex.** *J Cell Physiol* 1992, **151**:81-93.
FGF2 lacks a signal peptide but is known to act extracellularly. This study shows that FGF2 can be released via an exocytotic mechanism independent of the endoplasmic reticulum-Golgi pathway.
29. Miyakawa K, Hatsuzawa K, Kurokawa T, Asada M, Kuroiwa T, Imamura T: **A hydrophobic region locating at the center of fibroblast growth factor-9 is crucial for its secretion.** *J Biol Chem* 1999, **274**:29352-29357.
FGF9 lacks an amino-terminal signal sequence but is secreted from expressing cells. This study shows that nascent FGF9 polypeptides translocate into endoplasmic reticulum without peptide cleavage via a co-translational pathway in which both an amino terminus and a central hydrophobic domain is important.
30. Revest JM, DeMoerlooze L, Dickson C: **Fibroblast growth factor 9 secretion is mediated by a non-cleaved amino-terminal signal sequence.** *J Biol Chem* 2000, **275**:8083-8090.
FGF9 lacks a recognizable amino-terminal signal sequence, although it is efficiently secreted. This study shows that FGF9 enters the endoplasmic reticulum and traverses the Golgi complex in a similar manner to other constitutively secreted proteins. They demonstrate that the first 28 amino acids of FGF9 can function as an efficient non-cleaved signal peptide.
31. 5mallwood PM, Munoz-sanjuan I, Tong P, Macke JP, Hendry SH, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J: **Fibroblast growth factor (FGF) homologous factors: new members of the FGF family implicated in nervous system development.** *Proc Natl Acod Sci USA* 1996, **93**:9850-9857.
This study identifies a novel FGF-related subfamily, termed fibroblast growth factor homologous factors (FHF). FHFs lack a classical signal sequence and contain nuclear localization signals. FHFs are expressed in the developing and adult nervous systems, suggesting a role in nervous system development and function.
32. Yamamoto 5, Mikami T, Ohbayashi N, Ohta M, Itoh N: **Structure and expression of a novel isoform of mouse FGF homologous factor (FHF)-4.** *Biochim Biophys Acto* 1998, **1398**:38-41.
Identification of alternative amino terminal coding exon for FHF4/FGF14.
33. Munoz-Sanjuan I, 5mallwood PM, Nathans J: **Isoform diversity among fibroblast growth factor homologous factors is generated by alternative promoter usage and differential splicing.** *J Biol Chem* 2000, **275**:2589-2597.
Characterization of multiple isoforms of *Fhfs1-4/Fgfs 11-14*, generated through the use of alternative 5' exons. Isoforms show different subcellular distributions and distinct expression patterns in developing and adult mouse tissues.
34. Wang Q, McEwen DG, Ornitz DM: **Subcellular and developmental expression of alternatively spliced forms of fibroblast growth factor 14.** *Mech Dev* 2000, **90**:283-287.
Identification of isoforms of *Fhf4/Fgf14* that result from the alternative usage of two different first exons. Isoforms show different subcellular localization and distinct expression patterns in developing and adult mouse tissues. Expression was observed in migrating and post migratory neurons.
35. Antoine M, Reimers K, Dickson C, Kiefer P: **Fibroblast growth factor 3, a protein with dual subcellular localization, is targeted to the nucleus and nucleolus by the concerted action of two nuclear localization signals and a nucleolar retention signal.** *J Biol Chem* 1997, **272**:29475-29481.
Identification of an FGF3 CUG initiation site. This amino-terminal extended product is localized in both the nucleus/nucleolus and secretory pathway. The amino terminus contains both a signal sequence and a nuclear localization signal.
36. Hogan BL: **Morphogenesis.** *Cell* 1999, **96**:225-233.
Review article highlighting the roles of multigene families, such as *Fgfs*, *Bmps*, *Hedgehogs*, *Wnts* and *Egfs* in morphogenesis.
37. Martin GR: **The roles of FGFs in the early development of vertebrate limbs.** *Genes Dev* 1998, **12**:1S71-1586.
Review article summarizing data on the role of FGFs in vertebrate limb development. Demonstrates that FGFs play essential roles in signaling centers that control establishment, outgrowth and patterning of the limb bud.

38. Lee PL, Johnson DE, Cousens LS, Fried VA, Williams LT: **Purification and complementary DNA cloning of a receptor for basic fibroblast growth factor.** *Science* 1989, **245**:57-60.
This is the first cloning of an FGF receptor.

39. Johnson DE, Lee PL, Lu J, Williams LT: **Diverse forms of a receptor for acidic and basic fibroblast growth factors.** *Mol Cell Biol* 1990, **10**:4728-4736.
Identification of FGF receptor 1 variants which contain either two or three immunoglobulin-like domains. Identification of putative secreted forms of FGF receptor 1.

40. McKeehan WL, Wang F, Kan M: **The heparan sulfate-fibroblast growth factor family: diversity of structure and function.** *Prog Nucleic Acid Res Mol Biol* 1998, **59**:135-176.
This review presents models for FGF receptor activation by FGF and heparin. Also discussed are developmental roles for FGFs in liver and prostate development.

41. Miki T, Bottaro DP, Fleming TP, Smith CL, Burgess WH, Chan AM, Aaronson SA: **Determination of ligand-binding specificity by alternative splicing: two distinct growth factor receptors encoded by a single gene.** *Proc Natl Acod Sci USA* 1992, **89**:246-250.
Demonstration that FGFR alternative splicing can regulate ligand binding specificity.

42. Chellaiah AT, McEwen DG, Werner S, Xu J, Ornitz DM: **Fibroblast growth factor receptor (FGFR) 3. Alternative splicing in immunoglobulin-like domain III creates a receptor highly specific for acidic FGF/FGF-1.** *J Biol Chem* 1994, **269**:11620-11627.
Identification of an alternative splice form of FGFR3 with very restricted ligand binding properties.

43. Naski MC, Ornitz DM: **FGF signaling in skeletal development.** *Front Biosci* 1998, **3**:D781-D794.
Review article highlighting the functions of the *Fgfrs* with a focus on roles in skeletal development.

44. Yan G, Fukabori Y, McBride G, Nikolaropolous S, McKeehan WL: **Exon switching and activation of stromal and embryonic fibroblast growth factor (FGF)-FGF receptor genes in prostate epithelial cells accompany stromal independence and malignancy.** *Mol Cell Biol* 1993, **13**:4513-4522.
Demonstration that tumor progression can be accompanied by a change in *Fgfr* alternative splicing with consequent change in ligand binding specificity.

45. Orr-Urtreger A, Bedford MT, Burakova T, Arman E, Zimmer Y, Yayon A, Givol D, Lonai P: **Developmental localization of the splicing alternatives of fibroblast growth factor receptor-2 (FGFR2).** *Dev Biol* 1993, **158**:475-486.
Demonstration that alternative *Fgfr* splice forms are expressed preferentially in epithelial and mesenchymal lineages.

46. Gilbert E, Del Gatto F, Champion-Arnaud P, Gesnel MC, Breathnach R: **Control of BEK and K-SAM splice sites in alternative splicing of the fibroblast growth factor receptor 2 pre-mRNA.** *Mol Cell Biol* 1993, **13**:5461-5468.
Identification of the mechanism regulating tissue-specific usage of alternative splice forms of *Fgfr2*.

47. Avivi A, Yayon A, Givol D: **A novel form of FGF receptor-3 using an alternative exon in the immunoglobulin domain III.** *FEBS Lett* 1993, **330**:249-252.
Identification of alternative splice forms of *Fgfr3*.

48. Scotet E, Houssaint E: **The choice between alternative IIIb and IIIc exons of the FGFR-3 gene is not strictly tissue-specific.** *Biochim Biophys Acta* 1995, **1264**:238-242.
The less stringent alternative splicing choice between exons IIIb and IIIc of FGFR3 is in contrast with the very tissue-specific alternative splicing observed for FGFR2 in which epithelial cells use only the IIIb exon and fibroblasts use only the IIIc exon.

49. Ornitz DM, Xu J, Colvin JS, McEwen DG, MacArthur CA, Coulier F, Gao G, Goldfarb M: **Receptor specificity of the fibroblast growth factor family.** *J Biol Chem* 1996, **271**:15292-15297.
The first comparison of the relative mitogenic activity of a large number of FGFs on cells expressing a single alternatively spliced FGFR 1-3 or FGFR4.

50. Igarashi M, Finch PW, Aaronson SA: **Characterization of recombinant human fibroblast growth factor (Fgf-10) reveals functional similarities with keratinocyte growth factor (Fgf-7).** *J Biol Chem* 1998, **273**:13230-13235.
Demonstrates that the receptor-binding specificity of FGF10 is similar to that of the closely related FGF7.

51. Miki T, Fleming TP, Bottaro DP, Rubin JS, Ron D, Aaronson SA: **Expression cDNA cloning of the KGF receptor by creation of a transforming autocrine loop.** *Science* 1991, **251**:72-75.
First cloning of a receptor for FGF7/KGF.

52. MacArthur CA, Lawshé A, Xu J, Santos-Ocampo S, Heikinheimo M, Chellaiah AT, Ornitz DM: **FGF-8 isoforms activate receptor splice forms that are expressed in mesenchymal regions of mouse development.** *Development* 1995, **121**:3603-3613.
Identification of alternatively spliced forms of FGF8 and demonstration that these molecules bind the mesenchymal splice forms of *Fgfr2*.

53. Heikinheimo M, Lawshé A, Shackleford GM, Wilson DB, MacArthur CA: ***Fgf-8* expression in the post-gastrulation mouse suggests roles in the development of the face, limbs and central nervous system.** *Mech Dev* 1994, **48**:129-138.
Identification of temporal and spatial patterns of *Fgf8* expression in the developing mouse.

54. Crossley PH, Martin GR: **The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo.** *Development* 1995, **121**:439-451.
Identification of alternative splice forms of *Fgf8* and *Fgf8* expression in the apical ectodermal ridge.

55. Moscatelli D: **High and low affinity binding sites for basic fibroblast growth factor on cultured cells: absence of a role for low affinity binding in the stimulation of plasminogen activator production by bovine capillary endothelial cells.** *J Cell Physiol* 1987, **131**:123-130.
This paper challenges the role of cell surface heparan sulfate in FGFR activation but establishes a role for cell surface heparan sulfate in binding FGF.

56. Flaumenhaft R, Moscatelli D, Rifkin DB: **Heparin and heparan sulfate increase the radius of diffusion and action of basic fibroblast growth factor.** *J Cell Biol* 1990, **111**:1651-1659.
This paper demonstrates that the extracellular matrix can store FGF and limit its diffusion in tissues.

57. Mach H, Volkin DB, Burke CJ, Middaugh CR, Linhardt RJ, Fromm JR, Loganathan D, Mattsson L: **Nature of the interaction of heparin with acidic fibroblast growth factor.** *Biochemistry* 1993, **32**:5480-5489.
This paper establishes that FGF1 binds heparin at high density (one molecule every 4-5 saccharide units) and with high affinity (50-140 nM).

58. Herr AB, Ornitz DM, Sasisekharan R, Venkataraman G, Waksman G: **Heparin-induced self-association of fibroblast growth factor 2. Evidence for two oligomerization processes.** *J Biol Chem* 1997, **272**:16382-16389.
Presents analytical data that indicates that biologically active heparin octasaccharides can induce a monomer-dimer-tetramer assembly of FGF2.

59. Moy FJ, Safran M, Seddon AP, Kitchen D, Bohlen P, Aviezer D, Yayon A, Powers R: **Properly oriented heparin-decasaccharide-induced dimers are the biologically active form of basic fibroblast growth factor.** *Biochemistry* 1997, **36**:4782-4791.
Evidence that a *cis*-oriented FGF2 dimer is the minimal biologically active structural unit of FGF2 in the presence of a decasaccharide.

60. Ornitz DM, Yayon A, Flanagan JG, Svahn CM, Levi E, Leder P: **Heparin is required for cell-free binding of basic fibroblast growth factor to a soluble receptor and for mitogenesis in whole cells.** *Mol Cell Biol* 1992, **12**:240-247.
First study to show that heparin, FGF and an FGF receptor binding domain form a trimolecular complex in *vitro*. This paper also shows that this complex is essential for receptor activation on cells lacking heparan sulfate proteoglycan.

61. Rapraeger AC, Krufka A, Olwin BB: **Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation.** *Science* 1991, **252**:1705-1708.
The first study to demonstrate that cell surface heparan sulfate is directly involved in FGF cell signaling.

62. Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM: **Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor.** *Cell* 1991, **64**:841-848.
The first study to demonstrate that heparin is required for FGF to bind to an FGF receptor.

63. Lin XH, Buff EM, Perrimon N, Michelson AM: **Heparan sulfate proteoglycans are essential for FGF receptor signaling during *Drosophila* embryonic development.** *Development* 1999, **126**:3715-3723.
The first genetic evidence that heparan sulfate glycosaminoglycans are essential for fibroblast growth factor receptor signaling in a well defined

developmental context. These data support the model in which heparan sulfate facilitates FGF and/or FGF-FGFR oligomerization.

64. Szebenyi G, Fallon JF: **Fibroblast growth factors as multifunctional signaling factors.** *Int Rev Cytol* 1999, **185**:45-106.
This is a comprehensive review that highlights all aspects of FGF biology including cell signaling, receptor binding and activation and roles for FGFs in embryonic development.

65. Schlessinger J, Plotnikov AN, Ibrahimi OA, Eliseenkova AV, Yeh BK, Yayon A, Linhardt RJ, Mohammadi M: **Crystal structure of a ternary FGF-FGFR-heparin complex reveals a dual role for heparin in FGFR binding and dimerization.** *Mol Cell* 2000, **6**:743-750.
This paper presents a diffusion-based crystal structure which shows heparan fragments bound to both FGF and the FGFR in a head-to-head orientation.

66. Chellaiah A, Yuan W, Chellaiah M, Ornitz DM: **Mapping ligand binding domains in chimeric fibroblast growth factor receptor molecules. Multiple regions determine ligand binding specificity.** *J Biol Chem* 1999, **274**:34785-34794.
Identification of a second ligand-binding region on the FGF receptor.

67. Meyers EN, Lewandoski M, Martin GR: **An *Fgf8* mutant allelic series generated by Cre- and Flp-mediated recombination.** *Nat Genet* 1998, **18**:136-141.
First null, hypomorphic and conditional allele created for an FGF. This study reveals requirements for *Fgf8* during gastrulation, cardiac, craniofacial, forebrain, midbrain and cerebellar development.

68. Moon AM, Capecchi MR: **Fgf8 is required for outgrowth and patterning of the limbs.** *Nat Genet* 2000, **26**:455-459.
Demonstrates an essential role for *Fgf8* in limb bud development.

69. Lewandoski M, Sun X, Martin GR: **Fgf8 signalling from the AER is essential for normal limb development.** *Nat Genet* 2000, **26**:460-463.
Demonstrates an essential role for *Fgf8* in limb bud development.

70. Reifers F, Bohli H, Walsh EC, Crossley PH, Stainier DY, Brand M: ***Fgf8* is mutated in zebrafish *acerebellar* (ace) mutants and is required for maintenance of midbrain-hindbrain boundary development and somitogenesis.** *Development* 1998, **125**:2381-2395.
Demonstrates a role for *Fgf8* in midbrain hindbrain development in zebrafish by demonstrating genetic linkage between *Fgf8* and the *acerebellar* (*ace*) mutation. Demonstrates that *ace* is probably a null mutation in *Fgf8*.

71. Heisenberg CP, Brennan C, Wilson SW: **Zebrafish aussicht mutant embryos exhibit widespread overexpression of ace (fgf8) and coincident defects in CNS development.** *Development* 1999, **126**:2129-2140.
Identification of a genetic locus that may regulate *Fgf8* (*ace*) expression in zebrafish.

72. Hebert JM, Rosenquist T, Gotz J, Martin GR: **FGF5 as a regulator of the hair growth cycle: evidence from targeted and spontaneous mutations.** *Cell* 1994, **78**:1017-1025.
Demonstrates that a null allele in the *Fgf5* gene is allelic with the mouse *angora* mutation. Identifies an essential role for FGF5 in regulating the hair growth cycle.

73. Carlton MBL, Colledge WH, Evans MJ: **Crouzon-like craniofacial dysmorphology in the mouse is caused by an insertional mutation at the Fgf3/Fgf4 locus.** *Dev Dyn* 1998, **212**:242-249.
Identification of a retroviral integration in the intragenic region between *Fgf3* and *Fgf4* in the Bulgy-eye (Bey) mutant mouse. Expression of both *Fgf3* and *Fgf4* is up-regulated in the cranial sutures of Bey mice. Phenocopies some features of Crouzon syndrome.

74. White KE, Evans WE, O'Riordan JL, Speer MC, Econs MJ, Lorenz-Depiereux B, Grabowski M, Meitinger T, Strom TM: **Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23.** *Nat Genet* 2000, **26**:345-348.
First identification of a missense mutation in an FGF ligand in a human genetic disease. Autosomal dominant hypophosphataemic rickets (ADHR) is characterized by low serum phosphorus concentrations, rickets, osteomalacia, lower extremity deformities, short stature, bone pain and dental abscesses.

75. White KE, Jonsson KB, Carn G, Hampson G, Spector TD, Mannstadt M, Lorenz-Depiereux B, Miyauchi A, Yang IM, Ljunggren O, *et al.*: **The autosomal dominant hypophosphatemic rickets (ADHR) gene is a secreted polypeptide overexpressed by tumors that cause phosphate wasting.** *J Clin Endocrinol Metab* 2001, **86**:497-500.
FGF-23, the gene mutated in ADHR, encodes a secreted protein and its mRNA is abundantly expressed by several different oncogenic hypophosphatemic osteomalacia tumors.

76. Gecz J, Baker E, Donnelly A, Ming JE, McDonald-McGinn DM, Spinner NB, Zackai EH, Sutherland GR, Mulley JC: **Fibroblast growth factor homologous factor 2 (FHF2): gene structure, expression and mapping to the Borjeson-Forssman-Lehmann syndrome region in Xq26 delineated by a duplication breakpoint in a BFLS-like patient.** *Hum Genet* 1999, **104**:56-63.
Identification of linkage between FGF12/FHF2 and Borjeson-Forssman-Lehmann syndrome.

77. Chang Z, Meyer K, Rapraeger AC, Friedl A: **Differential ability of heparan sulfate proteoglycans to assemble the fibroblast growth factor receptor complex in situ.** *FASEB J* 2000, **14**:137-144.
First paper to demonstrate that tissue-specific heparan sulfate molecules can affect patterns of FGF and FGF receptor binding in situ.

78. Madiai F, Hackshaw KV, Chiu IM: **Characterization of the entire transcription unit of the mouse fibroblast growth factor 1 (*FGF-1*) gene. Tissue-specific expression of the FGF-1.A mRNA.** *J Biol Chem* 1999, **274**:11937-11944.
This paper defines the entire transcriptional unit of the mouse *Fgf1* gene and shows that heart is the most abundant source of Fgf1.A mRNA.

79. Abraham JA, Whang JL, Tumolo A, Mergia A, Friedman J, Gospodarowicz D, Fiddes JC: **Human basic fibroblast growth factor: nucleotide sequence and genomic organization.** *EMBO J* 1986, **5**:2523-2528.
First cloned member of the FGF family.

80. Brookes S, Smith R, Thurlow J, Dickson C, Peters G: **The mouse homologue of hst/k-FGF: sequence, genome organization and location relative to int-2.** *Nucleic Acids Res* 1989, **17**:4037-4045.
This paper idenitifies the mouse *Fgf4* gene and shows that it is within 20 kb of the *Fgf3* gene on chromosome 7.

81. Colvin JS, Feldman B, Nadeau JH, Goldfarb M, Ornitz DM: **Genomic organization and embryonic expression of the mouse fibroblast growth factor 9 gene.** *Dev Dyn* 1999, **216**:72-88.
Contains a detailed analysis of the embryonic expression patterns of *Fgf9*.

82. Chiu IM, Liu Y, Payson RA: **Isolation of yeast artificial chromosomes containing the entire transcriptional unit of the human FGF1 gene: a 720-kb contig spanning human chromosome 5q31.3—>q32.** *Concer Genet Cytogenet* 1998, **106**:1-10.
Localization of the *Fgf1* gene to the q31-q33 region of chromosome 5, a regions that includes a number of genes encoding growth factors, growth factor receptors, hormone/neurotransmitter receptors.

83. Cox RD, Copeland NG, Jenkins NA, Lehrach H: **Interspersed repetitive element polymerase chain reaction product mapping using a mouse interspecific backcross.** *Genomics* 1991, **10**:375-384.
A rapid method to generate and map inter-repeat polymerase chain reaction products using DNA from interspecific backcross mice.

84. Lafage-Pochitaloff M, Galland F, Simonetti J, Prats H, Mattei MG, Birnbaum D: **The human basic fibroblast growth factor gene is located on the long arm of chromosome 4 at bands q26-q27.** *Oncogene Res* 1990, **5**:241-244.
Chromosomal localization of human FGF2.

85. Mattei MG, Pebusque MJ, Birnbaum D: **Chromosomal localizations of mouse Fgf2 and Fgf5 genes.** *Momm Genome* 1992, **2**:135-137.
Mapping of *Fgf2* and *Fgf5* on mouse chromosome 4.

86. Kim HS, Crow TJ: **Human proto-oncogene Int-2/FGF-3 map position 11q13.3-q13.4.** *Chromosome Res* 1998, **6**:579.
Chromosomal localization of human *FGF3*.

87. Yoshida CM, Wada M, Satoh H, Yoshida T, Sakamoto H, Miyagawa K, Yokota J, Koda T, Kakinuma M, Sugimura T, *et al.*: **Human HST1 (HSTF1) gene maps to chromosome band 11q13 and coamplifies with the Int2 gene in human cancer.** *Proc Natl Acod Sci USA* 1988, **85**:4861-4864.
Mapping of human *FGF4* to chromosome 11q13. Both *FGF3* and *FGF4* were co-amplified in DNA from a stomach cancer and a vulvar carcinoma cell line.

88. Peters G, Kozak C, Dickson C: **Mouse mammary tumor virus integration regions int-1 and int-2 map on different mouse chromosomes.** *Mol Cell Biol* 1984, **4**:375-378.
Demonstration that MMTV can activate expression of FGF3 to initiate oncogenesis in mouse mammary epithelial cells.

89. Peters G, Brookes S, Smith R, Placzek M, Dickson C: **The mouse homolog of the *hst/k-FGF* gene is adjacent to int-2 and is**

activated by proviral insertion in some virally induced mammary tumors. *Proc Natl Acad Sci USA* 1989, **86**:5678-5682.
Identification of a close linkage between *Fgf3* and *Fgf4* on mouse chromosome 7. Demonstration that either or both *Fgf3* and *Fgf4* can be activated by mouse mammary tumor virus integration.

90. Nguyen C, Roux D, Mattei MG, de Lapeyriere O, Goldfarb M, Birnbaum D, Jordan BR: **The FGF-related oncogenes hst and int.2, and the bcl.1 locus are contained within one megabase in band q13 of chromosome 11, while the fgf.5 oncogene maps to 4q21.** *Oncogene* 1988, 3:703-708.
Identification of physical linkage between *Fgf3* and *Fgf4* on human chromosome 11.

91. deLapeyriere O, Rosnet O, Benharroch D, Raybaud F, Marchetto S, Planche J, Galland F, Mattei MG, Copeland NG, Jenkins NA, *et al.*: **Structure, chromosome mapping and expression of the murine Fgf-6 gene.** *Oncogene* 1990, **5**:823-831.
Localization of *Fgf6* on mouse chromosome 6.

92. Marics I, Adelaide J, Raybaud F, Mattei MG, Coulier F, Planche J, de Lapeyriere O, Birnbaum D: **Characterization of the HST-related FGF.6 gene, a new member of the fibroblast growth factor gene family.** *Oncogene* 1989, **4**:335-340.
Cloning of *FGF6* based on sequence similarity with *FGF4*. *FGF6* was mapped to chromosome 12p13.

93. Zimonjic DB, Kelley MJ, Rubin JS, Aaronson SA, Popescu NC: **Fluorescence in situ hybridization analysis of keratinocyte growth factor gene amplification and dispersion in evolution of great apes and humans.** *Proc Natl Acad Sci USA* 1997, **94**:11461-1146S.
Chromosomal localization of *Fgf7* sequences in human and great ape genomes indicates that amplification and dispersion occurred in multiple discrete steps during evolution.

94. Mattei MG, deLapeyriere O, Bresnick J, Dickson C, Birnbaum D, Mason I: **Mouse Fgf7 (fibroblast growth factor 7) and Fgf8 (fibroblast growth factor 8) genes map to chromosomes 2 and 19 respectively.** *Momm Genome* 1995, **6**:196-197.
Mouse *Fgf7* and *Fgf8* genes were mapped to chromosomes 2F-G and 19C3-D, respectively.

95. Payson RA, Wu J, Liu Y, Chiu IM: **The human Fgf-8 gene localizes on chromosome 10q24 and is subjected to induction by androgen in breast cancer cells.** *Oncogene* 1996, **13**:47-S3.
Mapping human *FGF8* to chromosome 10q24.

96. Mattei MG, Penault-Llorca F, Coulier F, Birnbaum D: **The human FGF9 gene maps to chromosomal region 13q11-q12.** *Genomics* 1995, **29**:811-812.
Chromosomal localization of human *FGF9* to 13q11-q12.

97. Mattei MG, De Moerlooze L, Lovec H, Coulier F, Birnbaum D, Dickson C: **Mouse fgf9 (fibroblast growth factor 9) is localized on chromosome 14.** *Momm Genome* 1997, **8**:617-618.

98. Emoto H, Tagashira S, Mattei MG, Yamasaki M, Hashimoto G, Katsumata T, Negoro T, Nakatsuka M, Birnbaum D, Coulier F, *et al.*: **Structure and expression of human fibroblast growth factor-10.** *J Biol Chem* 1997, **272**:23191-23194.
Cloning and mapping of human FGF10 to 5p12-p13. Demonstration that FGF10 is similar to FGF7 in both sequence and biological activity.

99. Crackower MA, Heng HH, Tsui LC: **Assignment of mouse fibroblast growth factor 10 (Fgf10) gene to the telomeric region of chromosome 13.** *Genomics* 1998, **53**:247-248.
Mapping *Fgf10* to mouse chromosome 13.

100. Verdier AS, Mattei MG, Lovec H, Hartung H, Goldfarb M, Birnbaum D, Coulier F: **Chromosomal mapping of two novel human FGF genes, FGF11 and FGF12.** *Genomics* 1997, **40**:151-154.
Mapping human *FGF11* and *FGF12* to chromosome 17p12-p13 and 3q28, respectively.

101. Hartung H, Lovec H, Verdier AS, Mattei MG, Coulier F, Goldfarb M, Birnbaum D: **Assignment of Fgf12 to mouse chromosome bands 16B1—>B3 by in situ hybridization.** *Cytogenet Cell Genet* 1997, **76**:185-186.
Mapping mouse *Fgf12* to chromosome 13B1-3.

102. Liu Y, Chiu IM: **Assignment of Fgf12, the human Fgf homologous factor 1 gene, to chromosome 3q29->3qter by fluorescence in situ hybridization.** *Cytogenet Cell Genet* 1997, **78**:48-49.
Mapping human *FGF12* to chromosome 3q29-3qter.

103. Lovec H, Hartung H, Verdier AS, Mattei MG, Birnbaum D, Goldfarb M, Coulier F: **Assignment of FGF13 to human chromosome band Xq21 by in situ hybridization.** *Cytogenet Cell Genet* 1997, **76**:183-184.
Mapping *FGF13* to human chromosome Xq21.

104. Xu J, Lawshe A, MacArthur CA, Ornitz DM: **Genomic structure, mapping, activity and expression of fibroblast growth factor 17.** *Mech Dev* 1999, **83**:165-78.
Expression patterns of *Fgf17* and *Fgf8* and demonstration that *Fgf17* has similar receptor specificity, genomic organization and alternative splicing to *Fgf8*.

105. Whitmore TE, Maurer MF, Sexson S, Raymond F, Conklin D, Deisher TA: **Assignment of fibroblast growth factor 18 (FGF18) to human chromosome 5q34 by use of radiation hybrid mapping and fluorescence in situ hybridization.** *Cytogenet Cell Genet* 2000, **90**:231-233.
Mapping *FGF18* to human chromosome 5q34.

106. Xie MH, Holcomb I, Deuel B, Dowd P, Huang A, Vagts A, Foster J, Liang J, Brush J, Gu Q, *et al.*: **FGF-19, a novel fibroblast growth factor with unique specificity for FGFR4.** *Cytokine* 1999, **11**:729-735.
Identification of human *Fgf19* and demonstration of *in vitro* biological activity.

107. Kirikoshi H, Sagara N, Saitoh T, Tanaka K, Sekihara H, Shiokawa K, Katoh M: **Molecular cloning and characterization of human FGF-20 on chromosome 8p21.3-p22.** *Biochem Biophys Res Commun* 2000, **274**:337-343.
Cloning of human *FGF20* and mapping to chromosome 8p21.3-p22. FGF20 is most closely related to FGF9 and FGF16. *FGF20* mRNA was detected in a colon cancer cell line and at lower levels in human fetal tissues and primary tumors.

108. Nishimura T, Nakatake Y, Konishi M, Itoh N: **Identification of a novel FGF, FGF-21, preferentially expressed in the liver.** *Biochim Biophys Acta* 2000, **1492**:203-206.
Cloning of *Fgf21* an *Fgf* most closely related to *Fgf19* and *Fgf23*.

109. Nakatake Y, Hoshikawa M, Asaki T, Kassai Y, Itoh N: **Identification of a novel fibroblast growth factor, FGF-22, preferentially expressed in the inner root sheath of the hair follicle.** *Biochim Biophys Acta* 2001, **1517**:460-463.
Identification of *Fgf22*, a homolog of *Fgf7* and *Fgf10*.

110. Miller DL, Ortega S, Bashayan O, Basch R, Basilico C: **Compensation by fibroblast growth factor 1 (FGF1) does not account for the mild phenotypic defects observed in FGF2 null mice.** *Mol Cell Biol* 2000, **20**:2260-2268.
The relatively mild phenotypic defects associated with loss of FGF2 led to the hypothesis that other FGFs partially compensate. However, FGF1-FGF2 double-knockout mice are viable and fertile and do not display any gross phenotypic defects. Thus *Fgf1* null mice do not have a detectable phenotype and lack redundancy with *Fgf2*.

111. Tobe T, Ortega S, Luna JD, Ozaki H, Okamoto N, Derevjanik NL, Vinores SA, Basilico C, Campochiaro PA: **Targeted disruption of the FGF2 gene does not prevent choroidal neovascularization in a murine model.** *Am J Pathol* 1998, **153**:1641-1646.
This study demonstrates that FGF2 is not required for the development of choroidal neovascularization.

112. Ortega S, Ittmann M, Tsang SH, Ehrlich M, Basilico C: **Neuronal defects and delayed wound healing in mice lacking fibroblast growth factor 2.** *Proc Natl Acad Sci, USA* 1998, **95**:5672-5677.
Mice lacking Fgf2 are viable, fertile and phenotypically indistinguishable from wild type. Although not essential for embryonic development, loss of FGF2 leads to defects in cortical neurogenesis and skin wound healing in mice.

113. Zhou M, Sutliff RL, Paul RJ, Lorenz JN, Hoying JB, Haudenschild CC, Yin M, Coffin JD, Kong L, Kranias EG, *et al.*: **Fibroblast growth factor 2 control of vascular tone.** *Nat Med* 1998, **4**:201-207.
Mice lacking Fgf2 are morphologically normal but display decreased vascular smooth muscle contractility, low blood pressure and thrombocytosis.

114. Schultz JE, Witt SA, Nieman ML, Reiser PJ, Engle SJ, Zhou M, Pawlowski SA, Lorenz JN, Kimball TR, Doetschman T: **Fibroblast growth factor-2 mediates pressure-induced hypertrophic response.** *J Clin Invest* 1999, **104**:709-719.
Identification of a role for FGF2 in the pathogenesis of cardiac hypertrophy.

115. Mansour SL: **Targeted disruption of int-2 (fgf-3) causes developmental defects in the tail and inner ear.** *Mol Reprod Dev* 1994, **39**:62-67; discussion 67-68.
The first member of the *Fgf* family to be knocked out in mice. Developmental defects were discovered in the inner ear and in outgrowth of the tail.

116. Feldman B, Poueymirou W, Papaioannou VE, DeChiara TM, Goldfarb M: **Requirement of FGF-4 for postimplantation mouse development.** *Science* 1995, **267**:246-249.

Fgf4 null embryos implant but die shortly thereafter. *Fgf4* null embryos cultured *in vitro* displayed severely impaired proliferation of the inner cell mass.

117. Fiore F, Sebille A, Birnbaum D: **Skeletal muscle regeneration is not impaired in Fgf6-/- mutant mice.** *Biochem Biophys Res Commun* 2000, **272:**138-143.
Mice lacking *Fgf6* have normal muscle regeneration. This is in contrast to the relatively severe phenotype noted by Floss et *al.* [118].

118. Floss T, Arnold HH, Braun T: **A role for Fgf-6 in skeletal muscle regeneration.** *Genes Dev* 1997, **11:**2040-2051.
Mice lacking *Fgf6* show a severe muscle regeneration defect with fibrosis and myotube degeneration.

119. Fiore F, Planche J, Gibier P, Sebille A, Delapeyriere O, Birnbaum D: **Apparent normal phenotype of Fgf6-/- mice.** *Int J Dev Biol* 1997, **41:**639-642.
Mice lacking *Fgf6* are phenotypically normal.

120. Guo L, Degenstein L, Fuchs E: **Keratinocyte growth factor is required for hair development but not for wound healing.** *Genes Dev* 1996, **10:**165-175.
Mice lacking *Fgf7* have a unexpectedly subtle phenotype affecting hair growth.

121. Qiao J, Uzzo R, Obara-Ishihara T, Degenstein L, Fuchs E, Herzlinger D: **FGF-7 modulates ureteric bud growth and nephron number in the developing kidney.** *Development* 1999, **126:**547-554.
Identification of a second phenotype in mice lacking *Fgf7*. The developing ureteric bud and mature collecting system of *Fgf7*-null kidneys is markedly smaller than wild type and have 30% fewer nephrons than wild-type kidneys.

122. Sun X, Meyers EN, Lewandoski M, Martin GR: **Targeted disruption of Fgf8 causes failure of cell migration in the gastrulating mouse embryo.** *Genes Dev* 1999, **13:**1834-1846.
This study identifies *Fgf8* as a gene essential for gastrulation and shows that signaling via FGF8 and/or FGF4 is required for cell migration away from the primitive streak.

123. Shanmugalingam S, Houart C, Picker A, Reifers F, Macdonald R, Barth A, Griffin K, Brand M, Wilson SW: **Ace/Fgf8 is required for forebrain commissure formation and patterning of the telencephalon.** *Development* 2000, **127:**2549-2561.
Demonstration that *Fgf8/Ace* is required for the development of midline structures in the zebrafish forebrain.

124. Colvin JS, Green RP, Schmahl J, Capel B, Ornitz DM: **Male to female sex reversal in mice lacking fibroblast growth factor 9.** *Cell* 2001, in press.
This study demonstrates that FGF9 is essential for testicular mesenchymal growth, cord formation, and Sertoli and Leydig cell differentiation. FGF9 may also regulate mesonephric cell migration into the male gonad.

125. Ohuchi H, Hori Y, Yamasaki M, Harada H, Sekine K, Kato S, Itoh N: **FGF10 acts as a major ligand for FGF receptor 2 IIIb in mouse multi-organ development.** *Biochem Biophys Res Commun* 2000, **277:**643-649.
Diverse phenotypes of mice lacking *Fgf10* closely resemble those of mice lacking *FGFR2b*. Major phenotypes include the absence of thyroid, pituitary, and salivary glands in addition to the previously described absence of limbs and lungs. Minor defects were observed in the formation of the teeth, kidneys, hair follicles, and digestive organs.

126. Min H, Danilenko DM, Scully SA, Bolon B, Ring BD, Tarpley JE, DeRose M, Simonet WS: **Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to *Drosophila* branchless.** *Genes Dev* 1998, **12:**3156-3161.
Mice lacking *Fgf10* exhibited perinatal lethality associated with complete absence of lungs. *Fgf10* null mice also lack limbs. There is some disagreement with Sekine *et al.* [127] as to whether the apical ectodermal ridge transiently forms.

127. Sekine K, Ohuchi H, Fujiwara M, Yamasaki M, Yoshizawa T, Sato T, Yagishita N, Matsui D, Koga Y, Itoh N, Kato S: **Fgf10 is essential for limb and lung formation.** *Nat Genet* 1999, **21:**138-141.
Mice lacking *Fgf10* die at birth due to the lack of lung development beyond the trachea. Mice lacking *Fgf10* also had complete truncation of the forelimbs and hindlimbs. In *Fgf10* null embryos, limb bud formation was initiated but outgrowth of the limb buds did not occur.

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6896–6900, August 1992
Medical Sciences

Large induction of keratinocyte growth factor expression in the dermis during wound healing

(fibroblast growth factors/fibroblast growth factor receptors/gene expression/epidermis)

SABINE WERNER*, KEVIN G. PETERS*, MICHAEL T. LONGAKER†‡, FRANCES FULLER-PACE§, MICHAEL J. BANDA†, AND LEWIS T. WILLIAMS*¶

*Cardiovascular Research Institute, Department of Medicine, Howard Hughes Medical Institute, †Laboratory of Radiobiology and Environmental Health, and ‡Department of Surgery, University of California, San Francisco, CA 94143-0724; and §Imperial Cancer Research Fund, Viral Carcinogenesis Laboratory, London, United Kingdom

Communicated by Richard J. Havel, April 20, 1992 (received for review January 28, 1992)

ABSTRACT **Recent studies have shown that application of basic fibroblast growth factor (basic FGF) to a wound has a beneficial effect. However, it has not been assessed whether endogenous FGF also plays a role in tissue repair. In this study we found a 160-fold induction of mRNA encoding keratinocyte growth factor (KGF) 1 day after skin injury. This large induction was unique within the family of FGFs, since mRNA levels of acidic FGF, basic FGF, and FGF-5 were only slightly induced (2- to 10-fold) during wound healing, and there was no expression of FGF-3, FGF-4, and FGF-6 detected in normal and wounded skin. High levels of FGF receptor 1 and FGF receptor 2 mRNA and low levels of FGF receptor 3 mRNA were found in both normal and wounded skin. No change in the levels of these transcripts was detected during wound healing. *In situ* hybridization studies revealed highest levels of KGF mRNA expression in the dermis at the wound edge and in the hypodermis below the wound. In contrast, mRNA encoding the receptor of this growth factor (a splice variant of FGF receptor 2) was predominantly expressed in the epidermis. These results suggest that basal keratinocytes are stimulated by dermally derived KGF during wound healing and implicate a unique role of this member of the FGF family in wound repair.**

Cutaneous injury initiates a complex series of biological processes that are involved in wound healing. These processes involve many different cell types in migration, proliferation and differentiation, removal of damaged tissue, and production of extracellular matrices (1). Extensive histological studies have provided descriptive information on the cellular events involved in inflammation and tissue repair; however, little is known about the mediators that initiate and sustain wound repair.

The local application of platelet-derived growth factor (PDGF) or basic fibroblast growth factor (bFGF) to wounds has been shown to accelerate dermal as well as epidermal wound healing (2–8). Therefore, it seemed likely that endogenous PDGF and fibroblast growth factor (FGF) are also involved in tissue repair. Whereas the induction of PDGF and PDGF receptor expression in wounds has been demonstrated (9), nothing is known about the role of endogenous FGF in wound healing. To gain insight into the contribution of FGFs *in vivo* to the healing of full-thickness wounds, we have investigated the mRNA expression of the seven members of the FGF family as well as their receptors (FGFR1–FGFR3) in normal skin and during wound healing. Our data show that acidic FGF (aFGF), bFGF, FGF-5, and particularly keratinocyte growth factor (KGF) are expressed in normal skin and induced upon injury. Whereas KGF was predominantly expressed in stromal cells below the wound and at the wound edge, receptors for KGF (KGFR) were detected in the epidermis. This suggests that a KGF-mediated paracrine interaction may be important for the migration and proliferation of epidermal keratinocytes seen during wound healing. These data provide a molecular basis for understanding the mechanisms that contribute to tissue repair and imply an important role of KGF in wound healing.

MATERIALS AND METHODS

Animal Care. Mice were housed and fed according to the guidelines set forth by the Committee on Animal Research of the University of California, San Francisco.

Wounding and Preparation of Wound Tissue. Full-thickness excisional wounds were created along the backs of 50 adult BALB/c F_1 mice. Six wounds were created on each animal, and skin biopsy specimens from 10 animals were obtained at each of the following times: 12 hr, 1 day, 5 days, and 7 days after wounding. Biopsy of the 60 wounds from the 10 animals resulted in ≈4 cm^2 of tissue. A similar amount of skin was removed from the back of unwounded control animals. Tissue taken for RNA isolation was immediately frozen in liquid nitrogen. Tissue for *in situ* hybridization was fixed overnight in 4% paraformaldehyde in phosphate-buffered saline and subsequently frozen in OCT compound (Miles).

RNA Isolation and RNase Protection Assay. For RNA isolation, fresh skin biopsy specimens were frozen in liquid nitrogen and used for RNA isolation as described (10). For RNase protection mapping of FGF and FGFR transcripts, DNA probes were cloned into the transcription vector pBluescript II KS(+) (Stratagene) and linearized. An antisense transcript was synthesized *in vitro* by using T3 or T7 RNA polymerase and [^{32}P]rUTP (800 Ci/mmol, Amersham; 1 Ci = 37 GBq). Samples of 50 μg^{-1} of total cellular RNA were hybridized at 42°C overnight with 100,000 cpm of the labeled antisense transcript. Hybrids were digested for 40 min at 30°C with RNases A and T1 as described (11). Protected fragments were separated on 5% polyacrylamide/8 M urea gels and analyzed by autoradiography. The same RNA preparations were used for all protection assays. The increase in FGF mRNA levels was quantitated by laser scanning densitometry of the autoradiograms.

DNA Templates. FGFs were as follows: 227-base-pair (bp) fragment corresponding to nucleotides 242–469 of the murine aFGF cDNA (12); 239-bp fragment corresponding to nucleotides 175–414 of the murine bFGF cDNA (12); 276-bp fragment corresponding to nucleotides 1021–1297 of the murine Int-2 (mammary tumor integration site 2; FGF-3) cDNA (13); 343-bp fragment corresponding to nucleotides 267–610 of the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FGF, fibroblast growth factor; aFGF and bFGF, acidic and basic FGFs; KGF, keratinocyte growth factor; FGFR, FGF receptor; KGFR, KGF receptor.

¶To whom reprint requests should be addressed.

murine Hst (human stomach cancer; FGF-4) cDNA (12); 303-bp fragment corresponding to nucleotides 1–303 of the murine FGF-5 cDNA (12); 304-bp fragment corresponding to nucleotides 406–710 of the murine FGF-6 cDNA (14); 201-bp fragment corresponding to nucleotides 23–224 of the murine KGF cDNA (F.F.-P. and C. Dickson, unpublished data).

FGF receptors were as follows: 361-bp fragment corresponding to nucleotides 793–1154 of the murine FGFR1 cDNA (15). This fragment encodes the complete extracellular third immunoglobulin-like domain of the published murine FGFR1 sequence (15); 298-bp fragment corresponding to nucleotides 2140–2438 of murine FGFR2 cDNA (16). This fragment encodes the amino-terminal end of the first kinase domain and the kinase insert; 430-bp fragment corresponding to nucleotides 1233–1663 of the murine FGFR3 cDNA (D. Ornitz and P. Leder; personal communication). This fragment encodes sequences from the middle of the third immunoglobulin-like domain of murine FGFR3 to the beginning of the first kinase domain.

***In Situ* Hybridization.** Antisense riboprobes were made by *in vitro* transcription with T3 and T7 RNA polymerases and ^{35}S-labeled UTP as described (11). Plasmids encoding the transmembrane region of FGFR1, the kinase I/kinase insert region of FGFR2, and the coding sequences of KGF (see above) were used as templates. For *in situ* hybridization, skin and wound tissues were prepared as described (17). *In situ* hybridization was performed on frozen sections as described (18). After hybridization, sections were coated with NTB2 nuclear emulsion (Kodak) and exposed in the dark at 4°C for 18 days. After development, the sections were counterstained with hematoxylin/eosin.

RESULTS

mRNA Expression of FGFRs in Normal and Wounded Skin. To investigate the mRNA expression of FGFRs in normal and wounded skin, we isolated RNA from excisional wounds at different intervals after wounding and performed RNase protection assays. For each time point, 60 wounds from 10 mice were excised and used for RNA isolation. Normal skin from nonwounded mice was used as a control. mRNA encoding three different FGF receptors (FGFR1, FGFR2, and FGFR3) was detected in normal and wounded skin (Fig. 1). Whereas FGFR1 and FGFR2 were expressed at high levels, only low levels of FGFR3 mRNA were found. This is based on the finding that the protection assays with the FGFR1 and FGFR2 probes were exposed for 16 hr, whereas the protection assay with the FGFR3 probe was exposed for 4 days. No change in the mRNA levels of FGFR1 and FGFR2 was detected during the process of wound healing. FGFR3 mRNA was slightly induced within 5 days after injury.

mRNA Expression of FGFs in Normal and Wounded Skin. To investigate the mRNA expression of different FGFs in normal and wounded skin, we performed RNase protection assays with the same RNA preparations that had been used for the expression analysis of FGFR transcripts (see above). Consistent with recent findings (19), mRNA encoding KGF was detected in normal skin (Fig. 2). During wound healing the expression of this mRNA was considerably increased (Fig. 2); the expression level of KGF transcripts was induced 9-fold within 12 hr after wounding and 160-fold within 24 hr (Fig. 3). In 7-day wounds, KGF mRNA levels were still 100-fold higher compared with the unwounded basal level.

Relative to KGF expression, much lower levels of aFGF, bFGF, and FGF-5 were found in normal and wounded skin (see Fig. 3 and autoradiogram exposure times in the legend of Fig. 2). Expression of aFGF and FGF-5 was induced 10-fold and 2-fold, respectively, within 24 hr after wounding (Fig. 3). Expression of bFGF peaked at day 5 after wounding, and expression levels were 4-fold higher after this period compared with the basal level. In contrast to the prolonged induction of KGF expression, aFGF, bFGF, and FGF-5 transcripts returned to the basal value within 7 days after wounding (Fig. 3). No expression of FGF-3 (Int-2), FGF-4 (Hst), and FGF-6 mRNA was detected in normal or wounded skin (data not shown). These results show that several members of the FGF family are expressed during wound healing and that KGF is the predominant FGF induced during this process.

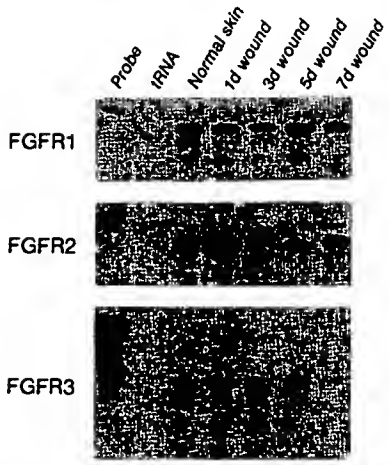


FIG. 1. Expression of FGFR mRNAs in normal and wounded skin. Total cellular RNA (50 μg) from normal and wounded mouse back skin was analyzed by RNase protection assay by using probes that hybridize to mRNA encoding FGFR1 (*Top*), FGFR2 (*Middle*), and FGFR3 (*Bottom*). Hybridization was performed under high-stringency conditions to avoid cross-hybridization with other FGFR mRNAs. The 361-nucleotide FGFR1 probe was complementary to the coding sequences of the complete third immunoglobulin-like domain of the published murine FGFR1 (15). The 298-nucleotide FGFR2 probe was complementary to sequences encoding the first kinase domain and the kinase insert of murine FGFR2 (16). The 430-nucleotide FGFR3 probe was complementary to sequences encoding the amino-terminal end of the third immunoglobulin-like domain, the transmembrane region, and the extracellular and intracellular juxtamembrane regions. The FGFR1 and FGFR2 gels were exposed for 16 hr, and the FGFR3 gel was exposed for 4 days. The time in days (d; 1, 3, 5, and 7) after injury is indicated at the top of each lane. Each hybridization probe (200–2000 cpm) was added to the lanes labeled "probe." They were used as a size reference and do not reflect the amounts of probe (100,000 cpm) that were added to all hybridization mixtures.

***In Situ* Hybridization of KGF and FGFR2 mRNA.** To determine the site of expression of KGF and FGFR2 during wound healing, we performed *in situ* hybridization on normal skin and on 1-day-old wounds. Consistent with the protection assay data, a significant induction of KGF mRNA expression was observed within 1 day after wounding, and transcripts encoding this growth factor were expressed at high levels in some cells below the wound (Fig. 4*D*) and at the wound edge (not shown). The observation that only certain cells in the dermis and hypodermis express high levels of KGF suggests that these cells represent a distinct cell population that might have an important function in wound healing.

The receptor for KGF was recently shown to be a splice variant of FGFR2 (20). Using a probe that detects all membrane-spanning variants of FGFR2, including the KGFR, we found mRNA encoding FGFR2 predominantly in the epidermis (Fig. 4*C*). By RNase protection assay we found that the specific splice variant of FGFR2 that is known to bind KGF is the predominant form of FGFR2 in the skin (data not shown). Therefore, the FGFR2 transcripts that we detected in the epidermis predominantly encode KGFRs. The same expression pattern of KGF and KGFR was observed at later

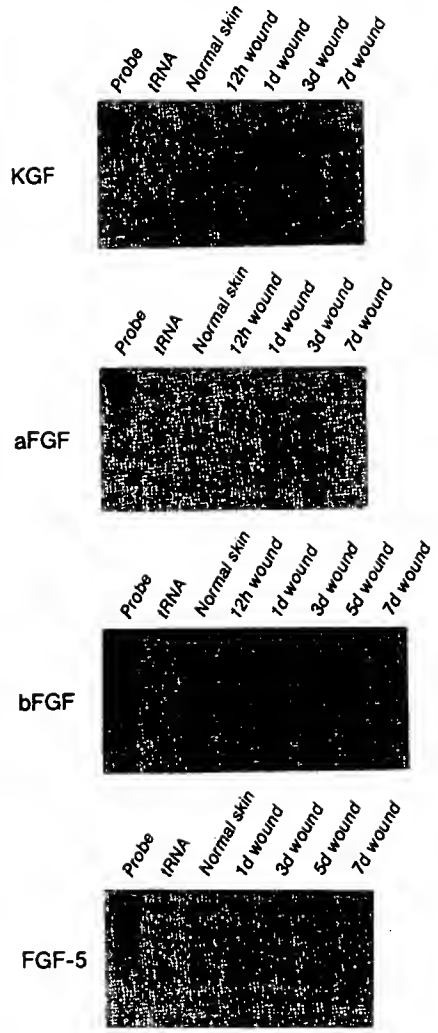


FIG. 2. mRNA expression of FGFs in normal and wounded skin. Total cellular RNA (50 μg) from normal and wounded back skin was analyzed by RNase protection assay with RNA hybridization probes complementary to mRNA encoding (from top to bottom) KGF, aFGF, bFGF, and FGF-5. Hybridizations were performed under high-stringency conditions to avoid cross-hybridizations with other FGF mRNAs. The same RNA preparations were used for all hybridizations of Fig. 1 and Fig. 2. The gels were exposed for 12 hr (KGF), 3 days (FGF-5), or 5 days (aFGF and bFGF). The time after injury is indicated on top of each lane: 12h, 12 hours; 1d, 3d, and 7d, 1, 3, and 7 days.

stages of wound healing (days 3–7 after injury), with KGF being expressed in the dermis and KGFR in proliferating keratinocytes which are in close proximity to the KGF-producing cells (data not shown). This expression pattern of KGF and FGFR2 in skin suggests that KGF produced in the dermis acts in a paracrine manner on epidermal keratinocytes.

DISCUSSION

FGFs comprise a family of polypeptide mitogens including aFGF (21), bFGF (22), FGF-3 (Int-2) (23), FGF-4 (Hst) (24, 25), FGF-5 (26), FGF-6 (27), and KGF (28). They are potent mitogens and chemotactic agents *in vitro* for many cells of mesenchymal and epithelial origin and, therefore, have the properties expected of wound cytokines. Furthermore, the

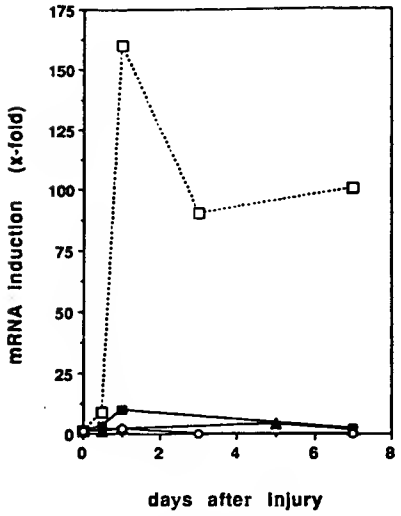


FIG. 3. Induction of aFGF, bFGF, and KGF mRNA expression after injury. The induction of aFGF (■), bFGF (▲), FGF-5 (○), and KGF (□) mRNA expression after injury is shown schematically. The mRNA induction compared with the basal level is shown as a function of time after injury. The increase in FGF mRNA levels was quantified by laser scanning densitometry of the autoradiograms. No expression of FGF-3, FGF-4, and FGF-6 was found in normal skin and within 7 days after injury.

direct application of bFGF to wounds has been shown to have a beneficial effect on accelerating wound healing (2–5).

To investigate the role of endogenous FGFs and FGFRs in wound healing, we have analyzed the mRNA expression of these growth factors in normal skin and during the process of wound healing. Our results show that several members of the FGF family are expressed in normal skin and are induced upon injury. In addition we show that at least three different FGFRs are expressed in normal and wounded skin. These data suggest that multiple FGF-mediated autocrine and paracrine stimulations contribute to the healing of wounds.

Our most striking finding is the extraordinary induction of transcripts encoding KGF within 24 hr after injury. Since KGF had been shown to be a specific and potent growth factor for epithelial cells (28), one might suggest that the induction of its expression during wound healing underlies the migration and proliferation of epithelial cells during this process. This hypothesis is supported by our finding that the induction of KGF expression precedes the onset of epithelial cell proliferation. Furthermore, it persists during the first 7 days after injury, which are characterized by significant migration and proliferation of epithelial cells (29). By *in situ* hybridization we detected expression of KGF mRNA predominantly in some cells below and at the edges of 1-day-old wounds (Fig. 4) and also in later stages of wound healing (days 3–7 after injury; data not shown). These cells most likely represent a distinct population of fibroblasts that might have an important role in wound healing. The detection of KGF transcripts in the dermis of normal and wounded skin is consistent with previous Northern blot results from other investigators who found KGF expression in the dermis but not in the epidermis of normal skin (19). A high-affinity receptor for KGF was recently identified on cultured keratinocytes but not on dermal fibroblasts (20). Therefore, it was suggested that KGF stimulates epithelial cell growth in a paracrine manner.

The receptor for KGF had been shown to be a splice variant of FGFR2, which binds KGF and aFGF with high affinity but bFGF only with low affinity (20, 30). In contrast, other known

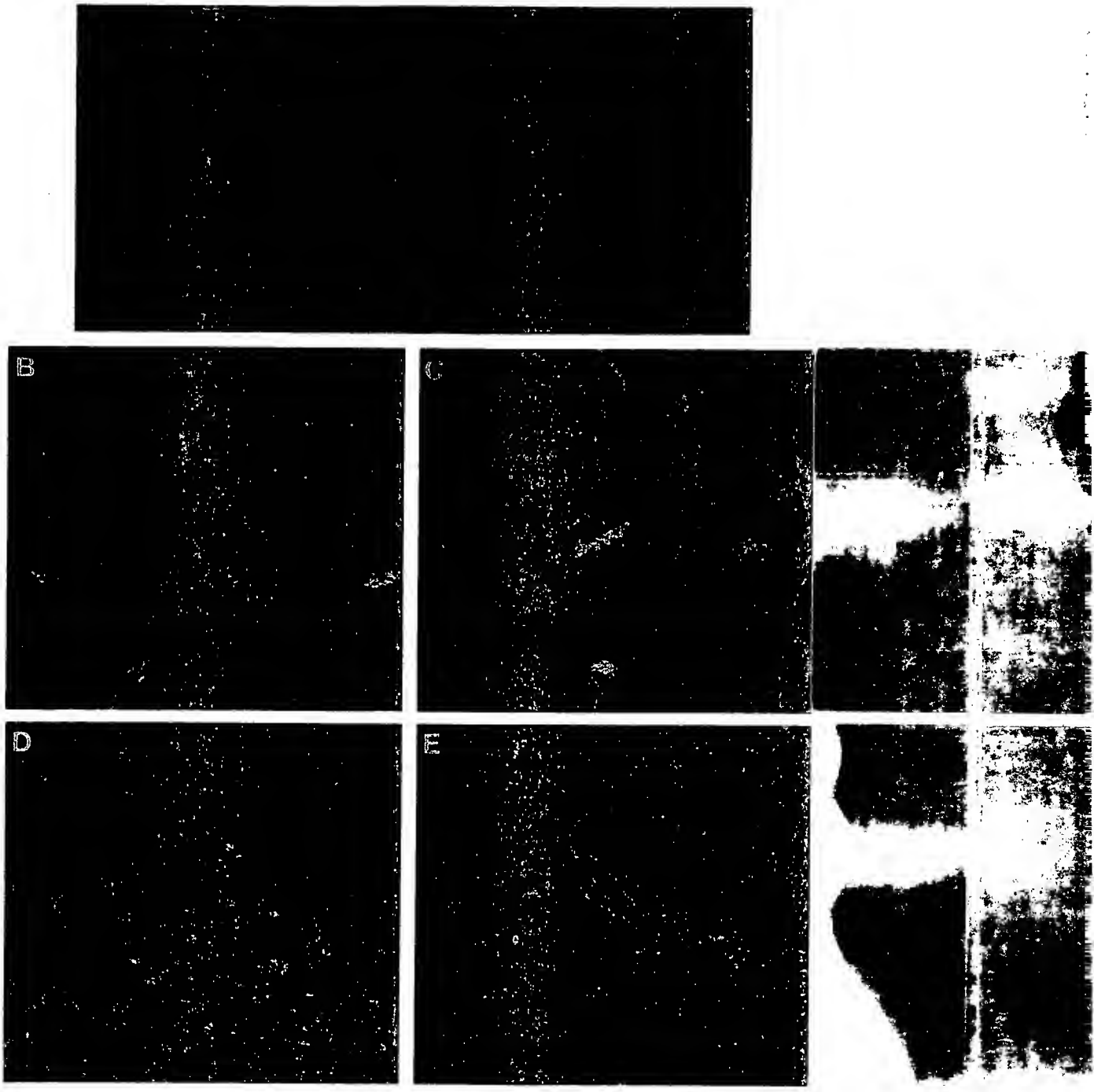


FIG. 4. Localization of FGFR2 and KGF mRNA in normal and wounded skin by *in situ* hybridization. (*A*) A hematoxylin/eosin stain of half of the wound. The arrowhead on the right indicates the region of normal skin where the photographs in *B* and *C* were taken. The arrow in the middle of the wound (left side of *A*) indicates the area where the photographs in *D* and *E* were taken. In *A*, the letters D, E, Es, and H indicate dermis, epidermis, eschar, and hair follicle, respectively. (*B–D*) (*B* and *C*) Sections from normal mouse back skin. (*D* and *E*) Sections from a 1-day-old wound. Sections were hybridized with ^{35}S-labeled riboprobes specific for KGF (*B* and *D*) and ^{35}S-labeled riboprobes specific for FGFR2 (*C* and *E*). The probes used for hybridization are described in *Materials and Methods*. The silver grains produced by the radioactive probe appear as white dots. They are indicated with arrows in *C* and *D*. In *B* and *C* the large bright nongranular areas represent artifacts of the autoradiography. Sections were counterstained with hematoxylin/eosin after hybridization. (*A*, ×45, *B–E*, ×170.)

FGFR2 splice variants bind aFGF and bFGF with high affinity but do not represent a receptor for KGF (30, 31). Our protection assay data demonstrate that the KGFR form is the predominant FGFR2 splice variant in skin (data not shown). Therefore, the FGFR2 transcripts which we detected in the epidermis of normal skin and wounds predominantly encode

receptors for KGF which should enable a paracrine stimulation of epidermal fibroblasts by dermally derived KGF.

In contrast to FGFR2, which is found predominantly in the epidermis, FGFR1 mRNA was expressed at highest levels in the dermis of normal and wounded skin (data not shown). A similar pattern of expression of FGFR1 and FGFR2 was recently observed in embryonic skin (32). The differential expression of the two FGFR genes suggests that the encoded proteins mediate different functions. We recently demonstrated that the predominant form of FGFR1 in the skin binds acidic and basic FGF with high affinity but does not represent a receptor for KGF (33). This shows that the major FGFR variants found in the dermis and epidermis have different ligand-binding specificities.

In addition to KGF, we also found induction of aFGF, bFGF, and FGF-5 mRNA expression during wound healing. However, these factors were only expressed at low levels and were induced to a much lesser extent compared with KGF. Among this group of factors expressed at low level, aFGF was induced to the greatest extent. This is of particular interest, since aFGF is the only FGF besides KGF that binds with high affinity to the KGFR (30). The induced expression of aFGF might further enhance the proliferation of keratinocytes during wound healing.

Expression of bFGF in skin is consistent with findings of other authors that show the encoded protein in the epidermis (34). In contrast, expression of aFGF and FGF-5 in skin has not been demonstrated so far. The mechanism of action of bFGF and aFGF in normal and wounded skin is presently unclear, since these growth factors lack a signal sequence and, therefore, are not secreted. One might speculate that these mitogens are released by damaged cells upon injury and subsequently act in an autocrine or paracrine manner. In contrast with aFGF and bFGF, the other members of the FGF family, including KGF, are clearly secreted mitogens.

With the exception of bFGF, induction of FGF gene expression seems to be an early event in wound healing, since highest levels of KGF, aFGF, and FGF-5 mRNA were found within 24 hr after injury. Expression of all FGFs subsequently declined, demonstrating the reversibility of the process. Therefore, it seems likely that specific control mechanisms initiate gene expression after injury and subsequently turn off expression after healing. A defect in FGF gene induction might likely be associated with impaired wound healing, whereas a defect in gene suppression could lead to the formation of hypertrophic scars or keloids. In addition, a prolonged induction of FGF gene expression might cause pathologic uncontrolled cell growth which might lead to tumor formation.

In summary, we have provided evidence that FGFs are expressed in normal skin and that their expression is induced during wound healing. The tremendous induction of KGF expression suggests that this mitogen is not only important in the physiological renewal of the epidermis but is of particular importance in the controlled epidermal cell proliferation seen in wound healing.

We thank Gail Martin, Jean Hebert, Jürgen Götz, Lee Niswander, and Jin-Kwan Han (University of California, San Francisco) for providing murine aFGF, bFGF, FGF-4, FGF-5, FGF-6, and Int-2 cDNAs and David Ornitz (Washington University School of Medicine, St. Louis) for murine FGFR3 cDNA. The excellent technical assistance of Xiang Liao and Linda Prentice is gratefully acknowledged. This work was supported by the National Institutes of Health Program of Excellence in Molecular Biology (HL-43821). K.G.P. is supported by a Physician Scientist Award from the National Institutes of Health. S.W. is supported by an Otto-Hahn fellowship from the Max-Planck-Gesellschaft, Germany.

1. Clark, R. A. F. (1985) *J. Am. Acad. Dermatol.* **13**, 701–710.
2. Greenhalgh, D. G., Sprugel, K. H., Murray, M. J. & Ross, R. (1990) *Am. J. Pathol.* **136**, 1235–1246.
3. Tsuboi, R. & Rifkin, D. B. (1990) *J. Exp. Med.* **172**, 245–251.
4. Hebda, P. A., Klingbeil, C. K., Abraham, J. A. & Fiddes, J. C. (1990) *J. Invest. Dermatol.* **95**, 626–631.
5. Klingbeil, C. K., Cesar, L. B. & Fiddes, J. C. (1991) *Prog. Clin. Biol. Res.* **365**, 443–458.
6. Lynch, S. E., Nixon, J. C., Colvin, R. B. & Antoniadis, H. N. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7696–7700.
7. Pierce, G. F., Mustoe, T. A., Senior, R. M., Reed, J., Griffin, G. L., Thomason, A. & Deuel, T. F. (1988) *J. Exp. Med.* **167**, 974–987.
8. Lynch, S. E., Colvin, R. B. & Antoniadis, H. N. (1989) *J. Clin. Invest.* **84**, 640–646.
9. Antoniadis, H. N., Galanopoulos, T., Neville-Golden, J., Kiritsy, C. P. & Lynch, S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 565–569.
10. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
11. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1985) *Nucleic Acids Res.* **12**, 7035–7056.
12. Hebert, J. M., Basilico, C., Goldfarb, M., Haub, O. & Martin, G. (1990) *Dev. Biol.* **138**, 454–463.
13. Mansour, S. L. & Martin, G. R. (1988) *EMBO J.* **7**, 2035–2041.
14. de Lapeyriere, O., Rosnet, O., Benharroch, D., Raybaud, F., Marchetto, S., Planche, J., Galland, F., Mattei, M.-G., Copeland, N. G., Jenkins, N. A., Coulier, F. & Birnbaum, D. (1990) *Oncogene* **5**, 635–643.
15. Reid, H. H., Wilks, A. F. & Bernard, O. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1596–1600.
16. Raz, V., Kelman, Z., Avivi, A., Neufeld, G., Givol, D. & Yarden, Y. (1991) *Oncogene* **6**, 753–760.
17. Rosenthal, A., Chan, S., Henzel, W. M., Haskell, C., Kuang, W.-J., Chen, E., Wilcox, J., Ullrich, A., Goeddel, D. & Routtenberg, A. (1987) *EMBO J.* **12**, 3641–3646.
18. Wilcox, J., Smith, K., Williams, L., Schwartz, S. & Gordon, D. (1988) *J. Clin. Invest.* **82**, 1134–1143.
19. Finch, P. W., Rubin, J. S., Miki, T., Ron, D. & Aaronson, S. (1989) *Science* **245**, 752–755.
20. Miki, T., Fleming, T. P., Bottaro, D. P., Rubin, J. S., Ron, D. & Aaronson, S. (1991) *Science* **251**, 72–75.
21. Jaye, M., Howk, R., Burgess, W., Ricca, G. A., Chiu, I., Ravera, M. W., O'Brien, S. J., Modi, W. S., Maciag, T. & Drohan, W. N. (1986) *Science* **233**, 541–545.
22. Abraham, J. A., Whang, J. L., Tumolo, A., Mergia, A., Friedman, J., Gospodarowicz, D. & Fiddes, J. C. (1986) *EMBO J.* **5**, 2523–2528.
23. Moore, R., Casey, G., Brookes, S., Dixon, M., Peters, G. & Dickson, C. (1986) *EMBO J.* **5**, 919–924.
24. Delli-Bovi, P., Curatola, A. M., Kern, F. G., Greco, A., Ittman, M. & Basilico, C. (1987) *Cell* **50**, 729–737.
25. Taira, M., Yoshida, T., Miyagawa, K., Sakamoto, H., Terada, M. & Sugimura, T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2980–2984.
26. Zhan, X., Bates, B., Hu, X. & Goldfarb, M. (1988) *Mol. Cell. Biol.* **8**, 3487–3495.
27. Marics, I., Adelaide, J., Raybaud, F., Mattei, M., Coulier, F., Planche, J., de Lapeyriere, O. & Birnbaum, D. (1989) *Oncogene* **4**, 335–340.
28. Rubin, J. S., Osada, H., Finch, P. W., Taylor, W. G., Rudikoff, S. & Aaronson, S. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 802–806.
29. Clark, R. A. F. (1991) in *Physiology, Biochemistry and Molecular Biology of the Skin*, ed. Goldsmith, L. A. (Oxford Univ. Press, New York), pp. 576–601.
30. Miki, T., Bottaro, D. P., Fleming, T. P., Smith, C. L., Burgess, W. H., Chan, A. M.-L. & Aaronson, S. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 246–250.
31. Dionne, C. A., Crumley, G., Bellot, F., Kaplow, J. M., Searfoss, G., Ruta, M., Burgess, W. H. & Schlessinger, J. (1990) *EMBO J.* **9**, 2685–2692.
32. Peters, K. G., Werner, S., Chen, G. & Williams, L. T. (1992) *Development* **114**, 233–243.
33. Werner, S., Duan, D.-S., de Vries, C., Peters, K., Johnson, D. E. & Williams, L. T. (1992) *Mol. Cell. Biol.* **12**, 82–88.
34. Schulze-Osthoff, K., Goerdt, S. & Sorg, C. (1990) *J. Invest. Dermatol.* **95**, 238–240.